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КЛІНІЧНА МЕДИЦИНА

CLINICAL MEDICINE

- Bezshapochniy S.B., Loburets V.V., Loburets A.V., Dzhirov O.R., Podovzhniy O.G.**
Peculiarities of nasal irrigation in acute viral rhinosinusitis
- Beliaiev E.V., Filimonov V.Yu., Dmitriev M.O., Zakalata T.R.**
Frequency, structure, and dynamics of adenitis development and related secondary dental deformations among young people
- Budnik T.V., Kvashnina L.V.**
Prospects for mineral metabolic disorders correction in children with recurrent state of urinary tract infection
- Burluka V.V., Ankin N.L., Denisenko V.N., Maksymenko M.A., Shepitko K.V.**
Choice of terms for surgical treatment of unstable pelvic ring in victims with polytrauma based on anatomy-functional assessment of the trauma severity
- Vesnina L.E., Boriak Kh.R., Sokolenko V.M.**
Assessment of the dietary energy intake of young people with normal weight and overweight
- Volosovets O.P., Kryuchko T.O., Kryvopustov S.P., Gonchar M.O., Volosovets A.O., Stetsyuk R.A., Loginova I.O., Khomenko V.E., Shcherbynska K.M., Verbytskyi I.V.**
Morbidity and prevalence of the nervous system diseases in children of Ukraine
- Garashova M.A.**
Postmenopausal period - as a risk factor for the development of the reproductive system tumors
- Gasimzade G.Sh.**
Methods of radiation diagnostics of complications in combined craniocerebral trauma and abdominal trauma
- Dobryanskyi D.O., Horinets I.O., Mentshikova A.O., Sodomora O.O., Matsyura O.I.**
Preconditions for early and late intraventricular hemorrhages in preterm very low birth weight infants
- Kyrian O.A., Dorofeyev A.E., Khaymenova G.S., Dorofeyeva A.A.**
Role of single nucleotide gene polymorphisms in the development of ulcerative colitis
- Koval O.I.**
Substantiation of a safe period of time for the oral cavity sanitation under general anesthesia in children aged 12-18 years
- Kostyrenko O.P., Melnyk V.L., Shevchenko V.K., Sylenko, Yu.I. Yeroshenko G.A.**
Application of nanocrystals in treatment of chronic apical periodontitis
- Lizogub V.G., Kramarova V.N., Polonska L.N., Kaminska T. M., Melnychuk I.O., Tyravskaya Y.V.**
Dietary correction of hyperinsulinemia and hemostasis parameters in overweight arterial hypertension patients
- Безшапочний С.Б., Лобурець В.В., Лобурець А.В., Джиров О.Р., Подовжній О.Г.**
Особливості іригації носової порожнини при гострих вірусних риносинуситах
- Беляєв Е.В., Філімонов В.Ю., Дмитрієв М.О., Закалата Т.Р.**
Частота, структура та динаміка розвитку адентії і пов'язаних з ними вторинних деформацій зубних рядів у осіб молодого віку
- Буднік Т.В., Квашніна Л.В.**
Перспективи корекції мінерального дизметаболізму в дітей із рекурентним перебігом інфекції сечової системи
- Бурлука В.В., Анкін Н.Л., Денисенко В.Н., Максименко М.А., Шепітько К.В.**
Вибір строків хірургічного лікування нестабільного тазового кільця у постраждалих з політравмою на основі анатомо-функціональної оцінки тяжкості травми
- Весніна Л.Е., Бор'як Х.Р., Соколенко В.М.**
Оцінка енергетичної цінності харчового раціону в осіб молодого віку з нормальною і підвищеною масою тіла
- Волосовець О.П., Крючко Т.О., Кривоустов С.П., Гончарь М.О., Волосовець А.О., Стецюк Р.А., Логінова І.О., Хоменко В.Є., Щербинська К.М., Вербицький І.В.**
Захворюваність та поширеність хвороб нервової системи у дітей України
- Гарашова М.А.**
Постменопаузальний період як фактор ризику розвитку пухлин органів репродуктивної системи
- Гасимзаде Г.Ш.**
Методи променевої діагностики ускладнень поєднаної черепно-мозкової і абдомінальної травми
- Добрянський Д.О., Горинець І.Б., Меньшикова А.О., Содомора О.О., Мацюра О.І.**
Передумови виникнення ранніх та пізніх внутрішньо-шлуночкових крововиливів у недоношених новонароджених з дуже малою масою тіла
- Кир'ян О.А., Дорофєєв А.Е., Хайменова Г.С., Дорофєєва А.А.**
Роль одонуклеотидних поліморфізмів генів у розвитку неспецифічного виразкового коліту
- Коваль О. І.**
Обґрунтування безпечного проміжку часу проведення санації порожнини рота під загальним знеболенням у дітей віком 12-18 років
- Костиренко О.П., Мельник В.Л., Шевченко В.К., Силенко Ю.І., Єрошенко Г.А.**
Застосування нанокристалів для лікування хронічного верхівкового періодонтиту
- Лизогуб В.Г., Крамарьова В.Н., Полонська Л.Н., Камінська Т.М., Мельничук І.О., Тиравська Ю.В.**
Дієтична корекція гіперінсулінемії, показників гемостазу у хворих на артеріальну гіпертензію з підвищеною масою тіла

- Marchenko A.V., Loban G.A., Petrushanko T.O., Chereda V.V., Faustova M.O., Ananieva M.M.**
The effect of the psycho-emotional stress on the state of microbiota of the gingival sulcus
- Naumenko A.N., Skaletsky Yu.N., Regan M.M., Didkovsky V.L.**
The scope of inpatient mortality due to safety incidents in domestic hospitals
- Nevoit G.V., Potiazhenko M.M., Mintser O.P.**
Assessment of the functional types of body mobilization based on a dynamic analysis of spectral indicators of heart rate variability and their classification
- Pyra L.V., Svistilnik R.V., Romanchuk K.Yu., Gordiichuk O.O., Smolko D.G.**
Clinical and epidemiological characteristics of acute bacterial meningitis in adults of Khmelnytskyi region
- Puzryov G.S., Lyakhovskyi V.I., Shepitko V.I., Sydorenko A.V.**
Morphological justification of the stepwise dosed balloon angioplasty application compared to standard methods in patients with diabetic foot
- Rodionova V.V., Karasova O.V., Bekh O.E., Tkachenko V.A., Gordiienko Iu.A.**
Novel non-invasive severity markers in idiopathic pulmonary fibrosis
- Rubtsov R.V.**
Peculiarities of the immune status in industrial workers with pneumoconiosis in combination with chronic obstructive pulmonary disease
- Sarafinjuk L.A., Syvak A.V., Radyoha R.V., Sarafinjuk P.V., Matseyko I.I.**
Peculiarities of heart rate variability indicators in track-and-field athletics with mesomorphic somatotype
- Safaraliev F.R.**
Optimized measures for correction of dental status in professional athletes
- Semeniuk L.M., Yuzvenko T.Y., Borodkin H.O., Kryzhanovskaya O.I.**
Determination of the risks of infertility in women with thyroid pathology and hypoandrogenic ovarian dysfunction
- Skrypnykov P.M., Khavalkina L.M., Korobeinikova Y.L., Korobeinikov L.S., Tymoshenko Yu.V.**
Rationale for the use of methods of microscopic crystallography in fractals of proteins and irrigation for the early diagnosis, treatment and prevention of inflammatory processes in the oral cavity
- Sokol V.K., Kolesnichenko V.A., Protsenko O.S.**
Retrospective analysis of primary forensic medical examinations of the lower extremities mechanical trauma
- Talash R.V.**
The most significant morphological features of third molars in adult persons according to orthopantomography
- 69 **Марченко А.В., Лобань Г.А., Петрушанко Т.О., Черета В.В., Фаустова М.О., Ананьєва М.М.**
Вплив психо-емоційного стресу на стан мікробіоти ясневої борозни
- 74 **Науменко О.М., Скалецький Ю.М., Риган М.М., Дідковський В.Л.**
Масштаби стаціонарної летальності пацієнтів через інциденти безпеки у вітчизняних лікарняних закладах
- 77 **Невойт Г.В., Потяженко М.М., Мінцер О.П.**
Оцінка функціональних типів мобілізації організму на підставі динамічного аналізу спектральних показників варіабельності ритму серця та їх класифікація
- 81 **Пипа Л.В., Свістільнік Р.В., Романчук К.Ю., Гордійчук О.О., Смолко Д.Г.**
Клініко-епідеміологічна характеристика гострих бактеріальних менінгітів у дорослих Хмельницької області
- 87 **Пузырьов Г.С., Ляховський В.І., Шепітько В.І., Сидоренко А.В.**
Морфологічне обґрунтування застосування поетапної дозованої балонної ангіопластики у порівнянні зі стандартною методикою у хворих з ішемічною формою синдрому діабетичної стопи
- 91 **Родіонова В.В., Карасьова О.В., Бех О.Е., Ткаченко В.А., Гордієнко Ю.А.**
Нові неінвазивні маркери тяжкості перебігу ідіопатичного фіброзу легень
- 96 **Рубцов Р.В.**
Особливості імунного статусу у промислових працівників, хворих на пневмоконіоз у поєднанні з хронічним обструктивним захворюванням легень
- 101 **Сарафинюк Л.А., Сивак А.В., Радьога Р.В., Сарафинюк П.В., Мацейко І.І.**
Особливості показників варіабельності серцевого ритму у легкоатлетів мезоморфного соматотипу
- 106 **Сафаралієв Ф.Р.**
Оптимізація заходів корекції стоматологічного здоров'я у професійних спортсменів
- 111 **Семенюк Л.М., Юзвенко Т.Ю., Бородкін Г.О., Крижановська О.І.**
Визначення ризиків формування безпліддя у жінок з патологією щитовидної залози і гіпоандрогеновою дисфункцією яєчників
- 115 **Скрипников П.М., Хавалкіна Л.М., Коробейнікова Ю.Л., Коробейніков Л.С., Тимошенко Ю.В.**
Обґрунтування використання методів мікроскопічної кристалографії білкових фракталів та іригації для ранньої діагностики, лікування та профілактики запальних процесів порожнини рота
- 120 **Сокол В.К., Колесніченко В.А., Проценко О.С.**
Ретроспективний аналіз первинних судово-медичних експертиз при механічній травмі нижніх кінцівок
- 125 **Талаш Р.В.**
Найбільш значимі морфологічні особливості третіх молярів у людей зрілого віку за даними ортопантомографії

Tarianyk K.A., Lytvynenko N.V., Oliinychenko V.K., Byslik T.V., Pochernyaev K.F.
Adaptation of polymerase chain reaction-restriction fragment length polymorphism method for polymorphism (rs2583988) analysis in alpha-synuclein gene

Tyazhelov O.A., Karpinska O.D., Goncharova L.D., Klymovytskyi F.V.
Features of the abduction mechanism work before and after total hip joint arthroplasty

Shumna T.Ye., Fedosieieva O.S., Zinchenko T.P.
Assessment of respiratory system's functional state in children with bronchial asthma and allergic rhinitis

Yareshko A. G., Kulish M. V.
Glucocorticoids as immunostimulators in pathogenetic therapy of tuberculosis

130 Таряник К.А., Литвиненко Н.В., Олійниченко Є.К., Буслик Т.В., Почерняєв К.Ф.
Розробка методу полімеразної ланцюгової реакції довжин рестрикційних фрагментів для аналізу поліморфізму (rs2583988) гена альфа-сінуклеїна

134 Тяжелов О.А., Карпінська О.Д., Гончарова Л.Д., Климовицький Ф.В.
Особливості роботи абдукційного механізму до та після тотального ендопротезування кульшового суглоба

140 Шумна Т.С., Федосєєва О. С., Зінченко Т. П.
Оцінка функціонального стану дихальної системи у дітей з бронхіальною астмою та алергічним ринітом

144 Ярешко А. Г., Куліш М. В.
Глюкокортикоїди як імуностимулятори в патогенетичній терапії туберкульозу

ЕКСПЕРИМЕНТАЛЬНА МЕДИЦИНА

Antonenko P.P., Zazharskyi V.V., Suslova N.I., Sklyarov P. M., Reshetnichenko O.P., Kostyuk V.K., Mylostyvyi R.V.
Efficacy of herbal essential oils at tetrahydropyridine induced hepatitis in laboratory rats

Borodach V.A., Schnaider S.A., O.V. Suslova, N.N. Savieliieva, L.V. Anisimova, Ye.K. Tkachenko¹
Protective effect of quercetin on the oral cavity tissues in rats in the presence of genotoxicant and alimentary deficiency of polyphenols

Vastyanov R.S., Dzygal O.F., Gorlitsyna O.A., Mykhailenko V.L., Lapshyn D.Ye., Nazarenko O.Ya.
Pathological morpho-functional dysintegration as the key pathogenetic mechanism of experimental liver cirrhosis

Hryhorieva O.A., Matvieishyna T.M., Topolenko T.A.
Dynamics of ATP-positive dendritic cells in rat's oropharyngeal submucosa after antenatal antigen administration

Gubar I.V., Apykhtina O.L., Kaminsky R.F., Chaikovskiy Yu.B., Yavorovskiy O.P., Sokurenko L.M.
Organotoxic effect of single intratracheal administration of lead nanoparticles of different sizes

Yeroshenko G.A., Shevchenko K.V., Lisachenko O.D., Vilhova O.V., Yakushko O.S., Skotarenko T.A., Bilash V.P.
Ultrastructural remodeling of rat submandibular glands in chronic ethanol intoxication

Zaiats O.R., Ozhogan Z.R., Ozhogan I.A.
Morphometric analysis of maxillary tubercles in children to determine the possibility of their use as a support for distalization

Knysh O.V., Pakhomov O.V., Pogorila M.S., Savinova O.M., Balak O.K.
Thermal cycling as a method for disintegration of bifidobacterium bifidum

EXPERIMENTAL MEDICINE

149 Антоненко П.П., Зажарський В.В., Суськова Н.І., Склярів П.М., Решетниченко О.П., Костюк В.К., Милостивий Р.В.

Ефективність рослинних ефірних олій за тетрагідропіридинового гепатиту в лабораторних щурів

154 Бородач В.А., Шнайдер С.А., Суськова О.В., Савельєва Н.Н., Анісімова Л.В., Ткаченко Є.К.
Захисний вплив флавоноїду кверцетину на стан тканин ротової порожнини щурів в умовах дії генотоксиканта і аліментарної недостатності поліфенолів

159 Вастьянов Р.С., Дзигал О.Ф., Горліцина О.А., Михайленко В.Л., Лапшин Д.Є., Назаренко О.Я.
Патологічна морфо-функціональна дезінтеграція як провідний патогенетичний механізм при експериментальному цирозі печінки

164 Григор'єва О.А., Матвейшина Т.М., Тополенко Т.А.
Динаміка АТФ-позитивних дендритних клітин в підслизовій основі ротової частини глотки щурів після внутрішньоутробного введення антигена

168 Губар І.В., Апіхтіна О.Л., Камінський Р.Ф., Чайковський Ю.Б., Яворовський О.П., Сокурєнко Л.М.
Органотоксичний ефект однократного інтратрахеального введення наночастинок свинцю різного розміру

175 Єрошенко Г.А., Шевченко К.В., Лисаченко О.Д., Вільхова О.В., Якушко О.С., Скотаренко Т.А., Білаш В.П.
Ультроструктурне ремоделювання піднижньощелепних слинних залоз щурів при хронічній інтоксикації етанолом

179 Заяць О.Р., Ожоган З.Р., Ожоган І.А.
Морфометричний аналіз горбів верхньої щелепи у дітей для визначення можливості їх використання в якості опори при дисталізації

184 Книш О.В., Пахомов О.В., Погоріла М.С., Савінова О.М., Балак О.К.
Термоцикловання як метод дезінтеграції bifidobacterium bifidum

- Maslova G.S., Skrypnyk I.M., Yeroshenko G.A.**
Morphological features of doxorubicin-induced liver damage associated with nonalcoholic steatohepatitis
- Mykytenko A.O., Yeroshenko G.A.**
Reaction of hemomicrocirculatory bed of rat liver and changes in the functional state of the nitric oxide cycle under the conditions of modeling alcoholic hepatitis
- Pshychenko V.V., Chebotar L.D., Larycheva O.M., Tsviach O.A., Anasevych Ya.N.**
Influence of pineal gland's hypofunction on the structure of visceral organs
- Seredynska N.M., Korniyenko V.I., Marchenko-Tolsta K.S., Bobrytska O.M., Ladohubets O.V., Duchenko K.A.**
Cardiotropic influence of synthetic and genetically-engineered suppressors in rats with experimental rheumatoid arthritis combined with arterial hypertension
- Stetsuk Ye.V., Akimov O.Ye., Shepitko K.V., Boruta N.V., Goltsev A.N.**
Role of nitric oxide in development of fibrotic changes in rats' testes after 270 day central deprivation of testosterone synthesis
- Tymoshenko I.O., Cherkasov E.V., Shepitko K.V.**
Morphometric assessment of structural changes in the duodenal wall of rats caused by skin burn injury under conditions of experimental diabetes
- Tymchenko M.Ye., Ivanova Yu.V., Gramatiu S.M., Zarutskiy Ya.L., Bilyi V.Ya.**
Microenvironment of intestinal anastomoses with comparative assessment of immune status in patients with small intestine anastomoses
- Tiron O.I., Appelhans O.L., Gunas V.I., Chereshniuk I.L., Lysenko D.A.**
Indices of the cell cycle in the thyroid gland after thermal burns of the skin when using solutions of lactoprotein with sorbitol or haes-lx 5 %
- Fedosieieva O.V.**
Morphogenesis of rat's thyroid gland in preweaning period after prenatal influence of staphylococcal toxoid
- Fik V.B., Paltov Ye.V., Kryvko Yu.Ya**
Ultrastructural organization features of periodontal tissues after twelve weeks of opioid influence
- Kharchenko A.V., Chernov V.S., Kharchenko N.V., Makarenko P.M., Denysovets I.V., Denysovets T. M., Myronenko S.G.**
The role of chronic gastritis among precancerous diseases of the stomach
- Schnaider S.A., Balega M.I., Zombor Ye.V., Semenov Ye.I., Tkachenko Ye.K.**
Study on the effect of the vitamin and mineral complex containing zinc L-aspartate on the periodontal condition of rats in the presence of periodontitis modeling
- Schnaider S.A., Humeniuk V.V., Horokhivskiy V.N., Yefremova O.V., Khristova M.T., Tkachenko Ye.K.**
Effect of hormone-active metabolites of cholecalciferol on the state of the oral cavity tissues in rats under the conditions of estrogen deficiency and traumatic stress
- 189 **Маслова Г.С., Скрипник І.М., Єрошенко Г.А.**
Морфологічні особливості доксорубіцин-індукованих уражень печінки на тлі неалкогольного стеатогепатиту
- 194 **Микитенко А.О., Єрошенко Г.А.**
Реакція гемомікроциркуляторного русла печінки та зміни в функціональному стані циклу оксиду азоту за умов моделювання алкогольного гепатиту
- 200 **Пшиченко В.В., Чеботар Л.Д., Ларичева О.М., Цвях О.О., Анасевич Я.М.**
Вплив гіпофункції епіфізу на структуру вісцеральних органів
- 205 **Серединська Н.М., Корнієнко В.І., Марченко-Толста К.С., Бобрицька О.М., Ладогубець О.В., Дученко К.А.**
Кардіотропний вплив імуносупресорів синтетичного і генно-інженерного походження у щурів за експериментального ревматоїдного артрити, поєданого з артеріальною гіпертензією
- 211 **Стецук Є.В., Акімов О.Є., Шепітько К.В., Борута Н.В., Гольцев А.Н.**
Роль оксиду азоту в розвитку фібротичних змін сім'яників щурів після 270 днів центральної депривації синтезу тестостерона
- 215 **Тимошенко І.О., Черкасов Е.В., Шепітько К.В.**
Морфометричний аналіз структурних змін стінки дванадцятипалої кишки щурів при опіковій травмі шкіри за умов експериментального цукрового діабету
- 220 **Тимченко М.Є., Іванова Ю.В., Граматюк С.М., Заруцький Я.Л., Білий В.Я.**
Мікрооточення кишкових анастомозів з порівняльною оцінкою імунного статусу у пацієнтів з анастомозами тонкої кишки
- 225 **Тірон О.І., Аппельханс О.Л., Гунас В.І., Черешнюк І.Л., Лисенко Д.А.**
Показники клітинного циклу в щитоподібній залозі після термічного опіку шкіри при застосуванні розчинів лактопротеїну з сорбітолом або haes-lx 5 %
- 230 **Федосєєва О.В.**
Морфогенез щитоподібної залози щурів молочного періоду після пренатальної дії стафілококового анатоксину
- 234 **Фік В.Б., Пальтов Є.В., Кривко Ю.Я.**
Особливості ультраструктурної організації тканин пародонта через дванадцять тижнів опіоїдного впливу
- 238 **Харченко О.В., Черно В.С., Харченко Н.В., Макаренко П.М., Денисовець І.В., Денисовець Т.М., Мироненко С.Г.**
Роль хронічного гастриту серед передракових захворювань шлунка
- 242 **Шнайдер С.А., Балега М.І., Зомбор Є.В., Семенов Є.І., Ткаченко Є.К.**
Вивчення впливу вітамінно-мінерального комплексу, що містить цинк L-аспарагінат, на стан пародонта щурів в умовах моделювання пародонтиту
- 247 **Шнайдер С.А., Гуменюк В.В., Горохівський В.Н., Єфремова О.В., Хрестова М.Т., Ткаченко Е.К.**
Вплив гормонально-активних метаболітів холекальциферолу на стан тканин ротової порожнини щурів в умовах естрогенної недостатності і травматичного стресу

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PECULIARITIES OF NASAL IRRIGATION IN ACUTE VIRAL RHINOSINUSITIS

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The article describes the problems of choosing a solution for the nasal cavity irrigation in acute viral rhinosinusitis. The purpose of the study was to establish the effectiveness of restoring the physiological function of nasal structures by adding to the traditional (3% NaCl solution) saline solution, 6% N-acetylcysteine solution in combination with classical treatment regimen of acute rhinosinusitis. It was found that this combination can effectively restore the mucociliary transport of the nasal mucosa and thus reduce the risk of complications or the migration of the disease into a bacterial form. As a result of the study, we concluded that the combination of N-acetylcysteine with saline solution in the treatment of acute viral rhinosinusitis and improving both the objective and subjective condition of patients.

Key words: acute viral rhinosinusitis, irrigation, N-acetylcysteine, saline solutions.

The work is a fragment of the research project "Development of new medical technologies in the diagnosis and treatment of the upper respiratory tract pathologies", state registration No. 0115U006761.

Acute rhinosinusitis is one of the most common diseases in the world. According to statistics, every seventh person in Europe is diagnosed with rhinosinusitis every year, and the number of detected cases is constantly increasing. Given the data of the European Position Paper on Rhinosinusitis and Nasal Polyps (EPOS 2020) [6], the incidence of acute rhinosinusitis has increased over the past 20 years almost 2 times and ranges from 6% to 15% of the population.

The mucociliary clearance is the first and most important line of airway protection in the respiratory system against destructive pathogens and harmful environmental factors, which requires a coordinated cilia beating and the appropriate composition and amount of mucus on the epithelium surface, which provides the mucus transport into the oropharynx. Changes in this process make a person susceptible to nasal diseases [11].

Nasal mucus is a heterogeneous adhesive viscoelastic material that is released as a product of goblet epithelial cells and mucous cells of the submucosal glands. It is divided into two layers: gel and sol, which has a low viscosity, close to the water or plasma viscosity. The viscosity of the gel is about 1,000 times higher than the viscosity of the sol [2]. It is believed that the nasal gel transport with viruses, bacteria, allergens that have settled on it, becomes possible only after the rupture of transverse disulfide bonds between glycoproteins [1].

The main factors in the respiratory diseases pathogenesis, including rhinosinusitis, include a violation of the mucociliary transport mechanisms [1], as well as with a decrease in the number of intact active ciliated cells and an increase in goblet cells [2].

The main generally accepted principle of treatment is the fastest recovery of physiological action of nasal structures. Irrigation of the nasal cavity with saline solutions is one of the most popular methods of treatment [9], which helps to improve both the subjective well-being of the patient and potentiate the recovery rate.

Acetylcysteine, due to the presence of sulfhydryl groups, protects mucous cells from free radicals both by direct reaction with them and by supplying cysteine for glutathione synthesis [3]. Nasal mucus becomes more dense and viscous. The mucolytic action of N-acetylcysteine allows NaCl to overcome the viscosity of the gel, i.e. serves as a mean of delivering salt to the mucous epithelium.

Many species of bacteria live in colonies and form protective biofilms, which are a factor in the microorganisms resistance. N-acetylcysteine has been shown to inhibit the formation of biofilms [4, 12].

There is controversy about the actual effectiveness of mucolytic agents prescription in the treatment of rhinosinusitis. They are usually prescribed in clinical practice to reduce the viscosity and improve the mucus clearance, which helps to restore the physiological mechanisms of the paranasal sinuses and nasal epithelial barrier [5, 8].

The purpose of the study was to substantiate the effectiveness of N-acetylcysteine administration in combination with hypertonic saline in the treatment of patients with acute viral rhinosinusitis.

Materials and methods. On the basis of ENT Department of Poltava Regional Clinical Hospital in 2019-2020, employees of the Department of Otorhinolaryngology and Ophthalmology of the Ukrainian Medical Stomatology Academy performed a study of the effectiveness of the drug Flu-Acyl Rino in patients with a clinical diagnosis of acute viral rhinosinusitis. A total of 56 patients were observed in the study, which were divided into two groups: experimental (29 patients) and control (27 patients).

Inclusion criteria: 1) Age – 18 years and older; 2) The clinical diagnosis of acute viral rhinosinusitis.

Exclusion criteria: 1) Allergy to N-acetylcysteine; 2) Any surgery in the nasal cavity in the last 3 months; 3) The diagnosis of systemic nasal disease (e.g. cystic fibrosis, Kartagener's syndrome); 4) Inability to give informed consent form due to consciousness impairment; 5) Inability to adhere to the prescribed treatment or the specified visits.

All study participants were included only after signing an informed consent form. We used a classic scheme for the treatment of patients in the control group, including: nasal lavage with hypertonic (3%) saline [10] 2 doses in each nostril 3 times a day, topical corticosteroids (Mometasone furoate) 2 doses in each nostril 2 times a day. Patients in the experimental group received classical therapy, in which the standard hypertonic saline solution was replaced by the drug Flu-Acyl Rino (3% hypertonic NaCl solution + 6% N-acetylcysteine) – administration in the form of 2 doses of spray in each nostril 3 times a day.

Assessment of treatment methods on the basis of subjective signs of general well-being was performed using the Sino-Nasal Outcome Test (SNOT-22) [7]. The patient's health status questionnaire was performed on the day of the first visit, as well as on days 3 and 7. Symptoms will be presented by the sum of all criteria on a 5-point scale for each in SNOT-22.

Objective data were collected using the Karl Storz, Storz telecam II, Hopkins II endoscope with 0° angle of view, during the endoscopic examination at scheduled visits.

The study of the mucociliary transport function in the nasal mucosa was performed using a saccharin test with sodium saccharin. A piece of saccharin up to 1 mm³ was applied to the inferior nasal concha 1 cm away from its anterior end. The time from the moment of indicator application on the mucous membrane of the nasal cavity to the sweet taste sensation in the mouth was recorded. This method allows to obtain an integrated assessment of the state of mucociliary transport, as the result of the study depends on the rheological properties of mucus and motor activity of the ciliated epithelium. To determine the “conditional” rate of mucociliary clearance of the nasal mucosa by saccharin test, control studies of this indicator were performed in 32 practically healthy volunteers without significant anomalies of intranasal structures, who underwent a single study.

Determination of the state of nasal respiration in the studied patients was carried out by measuring the nasal airway resistance (NAR) by the method of active posterior rhinomanometry. The study was performed according to the developed regulations twice for the entire period of observation: 1 – during hospitalization; 2 – on the 7th day.

Clinical trial data were statistically processed by the method of variation statistics using Student's test, determination of arithmetic mean values of indices, confidence intervals and probability values (p) was performed using the computer programs Microsoft Excel 2016 and Statistica 13. The data in the tables and figures are presented as mean values and their standard deviations ($M \pm \sigma$) and standard errors ($M \pm m$).

Results of the study and their discussion. At the time of the initial examination, all patients in the study groups had subjective and objective signs that are characteristic of the diagnosis: Acute viral rhinosinusitis. Symptoms that could indicate the transition of this stage of the disease to the form of bacterial rhinosinusitis with signs of mucositis were not observed in any case. If viscous mucus with signs of stagnation was detected in the area of the osteomeatal complex during endomicroscopy, the patients underwent computed tomography examination of the paranasal sinuses. No subjective and objective signs of purulent rhinosinusitis were detected in any of the studied patients. In patients who had undergone polynosotomy for inflammatory diseases of the paranasal sinuses at various times in the past, endoscopy included examination of the sinuses through existing artificial openings. However, in any of the cases no excessive exudation in the sinuses was observed.

The condition of the nasal mucosa, which was assessed by endoscopic examination, suggested that the degree of its edema and abnormal discharge did not completely obstruct the patency of the nasal sinuses. This, in our opinion, was the basis for the assertion of the correctness of the study of the mucociliary transport.

According to the data obtained during the saccharin test (fig. 1), the speed of the mucociliary transport on the first day was 24.75 ± 5.83 minutes, for the experimental group and 22.85 ± 5.58 minutes – for the control group; on the third day – 16.68 ± 3.76 minutes in the experimental group and 19.6 ± 3.71 min

in the control group; on the seventh day – 11.15 ± 1.66 min in the experimental group and 14.53 ± 2.82 min in the control group.

Thus, already on the 7th day of treatment was determined a statistically significant difference in the functioning of the mucociliary transport on the indicators of the saccharin test between patients of the experimental and control groups ($p = 0.000001$), and indicators of recovery of mucociliary clearance activity in the experimental group are more dynamic, which is a statistically significant difference. If we compare the indicators with the conditional norm – 13.17 ± 4.72 minutes (fig. 2), we can conclude about the normalization of motor activity of the cilia of the ciliated epithelium immediately after the end of the pathological factors manifested against the background of acute rhinosinusitis, and this allows us to consider it as one of the main criteria for clinical recovery. In almost healthy volunteers there was a tendency to increase the time of mucociliary clearance (MCC) on the background of active smoking.

Analysis of the dynamics of changes in MCC showed that the greatest impact on its level had the manifestations of acute inflammatory process in the nasal cavity and paranasal sinuses. Thus, in patients in whom acute viral rhinosinusitis had signs of polysinusitis or pansinusitis, i.e. the frontal and posterior groups of paranasal sinuses were involved in the viral process at once, mucociliary clearance index was the highest in the preliminary study. Its level was also the highest in the study carried out on the 7th day and significantly exceeded the level of mucociliary clearance of patients with limited acute rhinosinusitis. The level of mucociliary clearance was also affected by the presence in the patient of intranasal structure disorders, such as nasal septum deviation, hypertrophy of the posterior ends of the inferior nasal concha, and so on.

In patients of the 1st study group, the dynamics of MCC significantly differed in patients with acute polysinusitis. Thus, against the background of administration of 3% hypertonic NaCl solution with 6% N-acetylcysteine, already on the 7th day of the study, the MCC in the experimental group was significantly different from patients in the control group, receiving traditional therapy, and the rate of MCC recovery to normal values was significantly faster ($p \leq 0.05$).

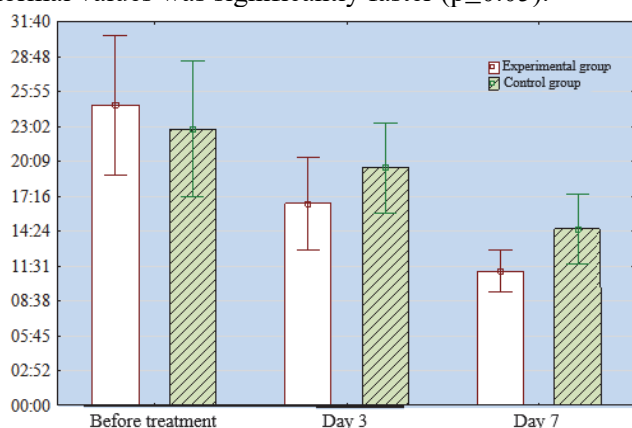


Fig. 1. Results of MCC study using saccharin test in patients of the studied groups; $M \pm \sigma$, min: sec.

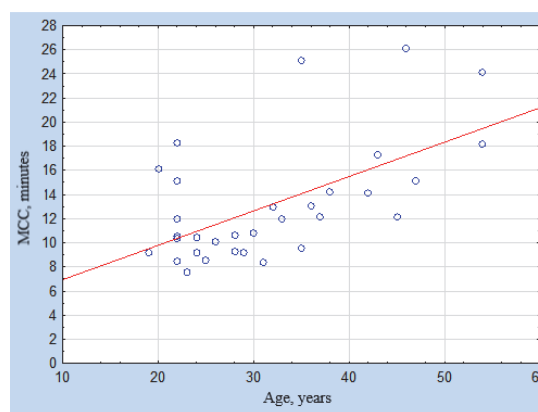


Fig. 2. Results of MCC study using saccharin test in almost healthy individuals.

For adequate interpretation of the obtained objective data, it is also necessary to correlate the results with the subjective condition of patients. The SNOT-22 scale allows to do it most effectively, as it covers all aspects of the patient's subjective well-being. This allows us to more accurately evaluate the objective data obtained during the visits, such as the saccharin test or posterior active rhinomanometry. It also allows for a more adequate assessment of the results obtained during endoscopic examination of the patient, and better assess the dynamics of the disease at all stages of treatment. This is crucial for achieving quality recovery of the patient because it allows timely response to changes and make decisions as quickly as possible on the treatment plan correction.

When performing the survey on SNOT-22 (fig. 3), on the first day there were worse results in the experimental group – 37.79 ± 8.22 points at 34.44 ± 7.45 points in the control one. On the third day, the obtained data were 33.55 ± 7.94 points in the experimental group and 29.89 ± 7.99 points in the control group. On the seventh day, the results for the experimental group were 11.66 ± 2.45 points and 12.67 ± 3.43 points in the control group. The data obtained in the first days of the disease do not reflect a significant improvement, which is the expected result for the start of treatment, as the disease process is in the acute phase and is not subject to significant, clinically significant correction.

From the fourth day onwards, patients' subjective well-being improved rapidly in both groups. On the 7th day of treatment, there was no statistically significant difference in SNOT-22 between patients in

the experimental and control groups ($p = 0.211626$). These data indicate a rapid reduction in the inflammatory process, as similar improvements in the disease are observed in patients during the convalescence period, the results of the survey on day 7 of the disease are expected changes in subjective well-being. This may indicate that it is the inflammatory process of the mucous membrane of the nasal cavity and paranasal sinuses that has a decisive influence on all the subjective signs observed by patients during the disease. The intoxication syndrome specifically attributed to the bacterial form of rhinosinusitis is not characteristic of the viral or post-viral form of the disease. Therefore, during treatment it is necessary to pay considerable attention to accelerating the inflammatory process convalescence, which in turn will not only improve the patient's well-being but also significantly accelerate recovery.

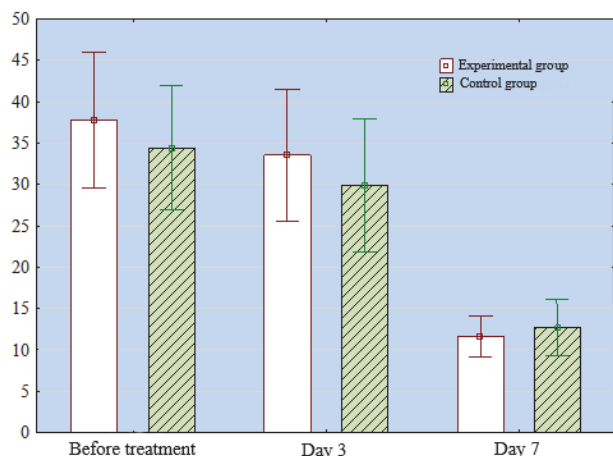


Fig. 3. The results of the patients' examination in the study groups; $M \pm \sigma$, SNOT 22 points.

decongestants was completely over, and had no effect on the results. Comparing the values of this indicator with the level of the conditional norm of nasal air resistance in healthy people, which corresponds to the value from 0.3 to 1.5 kPa/l *s, it can be concluded that on the 7th day of treatment in patients of both groups there is a normalization of nasal breathing index. Such a large range of indicators of the NAR level indicates a fairly high adaptive reserve of nasal breathing in different people, which depends not only on the architecture of the nasal cavity, but also on the physiological characteristics of the organism as a whole. The NAR magnitude was characterized by a significant amplitude, which indicates a fairly large adaptive reserve of the level of nasal breathing in different people, which significantly depends on the architecture of the nasal cavity. The difference in the A^1/A^2 coefficients between the control and experimental groups set out in table 1 has an improvement in the experimental and control groups, and does not have a statistically significant difference on day 7 of the study ($p = 0.294571$).

Rhinomanometry made it possible to study the dynamics of changes in the NAR in patients of the experimental and control groups, as shown in the table.

The results of the study of the NAR status by performing active posterior rhinomanometry revealed the magnitude of violations of this indicator in patients of different study groups. The greatest influence on the state of nasal breathing, in addition to the acute inflammatory process, had functional disorders of intranasal structures.

Due to technical aspects, not all patients of the studied groups underwent rhinomanometry before treatment and on the 7th day. At the time of the survey, the effect of previously used

Table 1

The level of NAR according to the results of active posterior rhinomanometry in patients of the studied groups (kPa/l*s)

Group	Before treatment		Day 7		Coefficient A^1/A^2
	n	$A^1 \pm m$	n	$A^2 \pm m$	
Experimental group	18	2.28 ± 0.13	14	1.27 ± 0.11	1.79
Control group	16	2.08 ± 0.09	11	1.41 ± 0.07	1.47

When reviewing the materials, it was noted that the indicators of rhinomanometry correlated with the changes obtained during endoscopic examinations of the nasal cavity, so we can talk about improving the objective state of its physiological functions. To determine the effectiveness of the topical action of the drugs used on the mucous membrane of the nasal cavity and to control the possibility of side effects, all patients underwent endoscopic examination at different times. In none of the cases any local manifestations of allergy or irritant effects of the studied drugs were observed.

During this study, we reported one case of a mild allergic reaction to the drug Flu-Acyl Rino in the form of a rash on the skin of single pink blisters, accompanied by itching. This patient was immediately excluded from this study and treated according to a standard protocol that takes into account allergic reactions to drugs. The effects of the allergic reaction completely disappeared after discontinuation of the drug the next day and then no longer bothered the patient.

The results of the study of mucociliary transport confirm the thesis that during the active inflammatory process of the mucous membrane, the significant gel viscosity violates the rate of mucus drainage containing pathological agents, and in turn prolongs inflammation of the mucous membrane as

described in Kunelska N.L. [2]. The results of the saccharin test confirm that the property of N-acetylcysteine to break the disulfide cross-links between glycoproteins, which in turn reduces the mucus viscosity and facilitates drainage of the nasal cavity, is of paramount importance to reduce the inflammatory process as reflected in the work Aldini G., Altomare A. et.al [3].

The results of the assessment of the patients' condition correlate with the results of clinical studies using the mucolytic agent N-acetylcysteine, which are described in the work of Bahtouee M., Monavarsadegh G. et.al. [4] and in our previous study on the treatment of acute rhinosinusitis [1]. Also, given the correlation between the results of well-being in patients in our study and the work of Macchi A, Terranova P, Castelnovo P. [5], the presented method of treatment will help optimize approaches to the choice of therapy, as it will allow less frequent use of systemic non-steroidal anti-inflammatory drugs, instead of choosing topical forms of drugs that have a better safety profile to improve the subjective symptoms in patients with acute rhinosinusitis.

Conclusions

After the study, it can be concluded that when using a hypertonic solution in combination with N-acetylcysteine (Flu-Acyl Rino) in the experimental group there was an objective indicators improvement (statistically significant difference on the 7th day of treatment according to the saccharin test, $p = 0.000001$), both the patient's subjective well-being and objective treatment outcomes improved, which in turn contributed to a more effective recovery of the patient compared to classical therapy, which uses saline solutions without additional components. Based on the study, we can recommend Flu-Acyl Rino as a front-line therapy for the treatment of acute viral rhinosinusitis in combination with standard therapy, to obtain a more pronounced effect of restoring the physiological function of the nasal structures.

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Реферати

ОСОБЛИВОСТІ ІРИГАЦІЇ НОВОЇ ПОРОЖНИНИ ПРИ ГОСТРИХ ВІРУСНИХ РИНОСИНУСИТАХ

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У статті описані проблеми вибору розчину для зрошення порожнини носа при гострих вірусних риносинуситах. Метою дослідження була перевірка ефективності відновлення фізіологічної роботи носових структур при додаванні до традиційного (3% розчин NaCl) сольового розчину, 6% розчину N-ацетилцистеїну в поєднанні з класичною схемою терапії гострих риносинуситів. Було встановлено, що дана комбінація дозволяє ефективно відновити роботу мукоциліарного транспорту слизової оболонки носа і таким чином зменшити ризики виникнення ускладнень процесу або переходу захворювання в бактеріальну форму. В

ОСОБЕННОСТИ ИРРИГАЦИИ НОВОЙ ПОЛОСТИ ПРИ ОСТРЫХ ВИРУСНЫХ РИНОСИНУСИТАХ

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В статье описаны проблемы выбора раствора для орошения полости носа при острых вирусных риносинуситах. Целью исследования была проверка эффективности восстановления физиологической работы носовых структур при добавлении к традиционному (3% раствор NaCl) солевому раствору, 6% раствора N-ацетилцистеина в сочетании с классической схемой терапии острых риносинуситов. Было установлено, что данная комбинация позволяет эффективно восстановить работу мукоцилиарного транспорта слизистой оболочки носа и таким образом уменьшить риски возникновения осложнений процесса или перехода заболевания в

результаті дослідження ми зробили висновок про ефективність комбінації N-ацетилцистеїну з сольовим розчином в лікуванні гострого риносинуситу та покращенні як об'єктивного, так і суб'єктивного стану пацієнтів.

Ключові слова: гострий вірусний риносинусит, іригація, N-ацетилцистеїн, сольові розчини.

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бактериальную форму. В результате исследования мы пришли к выводу об эффективности комбинации N-ацетилцистеина с солевым раствором в лечении острого риносинусита и улучшении как объективного, так и субъективного состояния пациентов.

Ключевые слова: Острый вирусный риносинусит, ирригация, N-ацетилцистеин, солевые растворы.

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FREQUENCY, STRUCTURE, AND DYNAMICS OF ADENTIA DEVELOPMENT AND RELATED SECONDARY DENTAL DEFORMATIONS AMONG YOUNG PEOPLE

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As part of the epidemiological survey study on dental health in young people, the data of clinical dental examination of 137 boys and 142 girls, the students of dental faculty of Vinnytsya National Pirogov Memorial Medical University, were analyzed. For comparative analysis of the structure of adentia the data of epidemiological study held in 2010 on the the Department of Orthopedic Dentistry were used. Statistical processing of the results was carried out in the Statistica 6.0 licensed package. The comparison of structure and frequency of adentia of young population in 2010 and 2019 revealed a decrease of frequency of this pathology. Change of the structure of adentia toward the relief of orthopedic pathology due to the significant decrease of adentia in the form of edentulous areas located posterior to the remaining natural teeth according to the topography should also be emphasized. A significant frequency of secondary deformations in the examined group with the prevalence of horizontal form was identified. In the structure of adentia there is a ninefold prevalence of adentia in the posterior area above the anterior one, a significant prevalence of adentia of the lower jaw.

Key words: missing teeth, epidemiology, secondary deformations.

The work is a fragment of the research project "Optimization of diagnostics, orthopedic treatment and prevention of jaw and facial system pathology", state registration No. 0119U103951.

In all age groups adentia occupies one of the dominant places in the structure of dental diseases [4]. Pulling teeth out in itself reduces the effectiveness of the masticatory function and, much more importantly, affects the morphology, development, and function of the entire dentition system [1]. At least one tooth pulled out triggers a whole cascade of changes and adjustments aimed at offsetting the loss of dentition integrity. These changes affect both the local adaptive mechanisms and the general ones and also cause psycho-emotional disorders [6].

Identifying indicators of the quality of life in dentistry as general indicators of a comprehensive assessment of the patient's condition [8] is often used to evaluate the effectiveness of treatment of the dentition system pathology, including adentia [12]. A great number of patients pay attention to function declining, disturbance of emotional and social well-being [10]. Concerns about appearance and behavior change aimed at masking defects of dentition are noted [13]. Aesthetics are the dominant motivating factor that encourages a patient to receive orthopedic or orthodontic dental care.

In adentia cases, there is a local functional sub- or decompensation of periodontal tissue of teeth around the defects of dental arches or opposite teeth. Along with inflammatory-destructive diseases of periodontal tissue that destroy its structure, missing tooth inevitably lead to the emergence of secondary deformations of dental arches [2].

The purpose of the study was to determine the frequency, structure, and dynamics of the development of adentia and related secondary deformations of dental arches among young people.

Material and methods. As a part of the epidemiological survey of dental health of young people, the data from clinical dental examination of 137 boys aged 17 to 21 years and 142 girls aged 16 to 20 years, the students of dental faculty of Vinnytsya National Pirogov Memorial Medical University, were analyzed.

The results of clinical examination were entered into a specially designed "patient's examination dental record", which displayed the whole spectrum of dental status.

The types of secondary deformations were determined according to the classification of Havrylov E. I. (1966) and Lebedenko N. Yu. (2007) [2]; dentition defects were determined according to Kennedy classification [1].

Determination of the minimum sufficient number of surveyed was carried out according to the recommendations of WHO [15].

For the comparative analysis of the structure of adentia, the data from epidemiological survey conducted in 2010 on the basis of the Department of Orthopedic Dentistry of Vinnytsya National Pirogov Memorial Medical University were used [7].

Statistical processing of the results was carried out in the "Statistica 6.0" licensed package.

Results of the study and their discussion. The epidemiological survey of frequency and structure of adentia and related secondary deformations will help to identify the need for dental care in various dental fields. According to the WHO Guidelines (1989), the issue of epidemiology of dental diseases needs to be updated and defined every five years [15].

Epidemiological survey data on the frequency of adentia in 2019 and 2010 are presented in table 1.

Table 1

The frequency of adentia emergence

Year of research	Gender	Adentia frequency
2019	overall (n=279)	14.0 % (n=39)
	boys (n=137)	6.8 % (n=19)
	girls (n=142)	7.2 % (n=20)
	P _{b-g}	>0.05
2010 [7]	overall (n=150)	33.3 % (n=50)
P _{ov2019-2010}		<0.001

In the survey we identified 39 individuals with adentia, which is 14.0 % of the total number of surveyed. The frequency of adentia had almost an equal gender distribution: boys with adentia accounted for 19 individuals (6.8%), girls with adentia accounted for 20 individuals (7.2 %). The results of epidemiological survey conducted in 2010 [7] showed the following frequency of adentia: 50 individuals (33.3 % of the total number of surveyed). So at the present stage there was a 2.37-fold ($p < 0.001$) decrease in the overall frequency of adentia.

This result could be associated with the improvement of therapeutic care and primary prevention of dental diseases.

The structure of adentia, subject to topography, according to epidemiological surveys conducted in 2019 and 2010 is presented in table 2.

Table 2

The structure of adentia under Kennedy classification

Year of research	Gender	Classes under Kennedy classification			
		1	2	3	4
2019	overall (n=39)	0 % (n=0)	5.1 % (n=2)	87.2 % (n=34)	7.7 % (n=3)
	boys (n=19)	0 % (n=0)	5.3 % (n=1)	84.2 % (n=16)	10.5 % (n=2)
	girls (n=20)	0 % (n=0)	5.0 % (n=1)	90.0 % (n=18)	5.0 % (n=1)
	P _{b-g}	>0.05	>0.05	>0.05	>0.05
2010 [7]	overall (n=50)	12.0 % (n=18)	16.0 % (n=24)	66.0 % (n=99)	6.0 % (n=9)
P _{ov2019-2010}		<0.05	>0.05	<0.05	>0.05

According to Kennedy classification, the structure of adenitis, subject to topography, was as follows: Class I – 0 %, Class II – 5.1 %, Class III – 87.2 %, Class IV – 7.7 %. It should be noted that there were no gender differences in the structure of adentia ($p > 0.05$).

A 2010 survey [7] showed the following distribution of adentia subject to topography: Class I – 12.0 %, Class II – 16.0 %, Class III – 66.0 %, Class IV – 6.0 %. Data comparison indicated a significant ($p < 0.05$) reduction of bilateral posterior defects and an increase ($p < 0.05$) of bounded posterior defects of the lateral section of dental arches and the relative permanence of frequency of dental arches defects in the anterior area.

The frequency of emergence of secondary deformations and the distribution of secondary deformations is presented in table 3.

Among individuals with adentia secondary deformations were determined in 25 persons, which made 64.1 %. Among them vertical deformations were detected in 4 individuals – 16.0 %; horizontal deformations were detected in 15 individuals – 60.0 %; mixed deformities were detected in 6 individuals – 24.0 %. Differences in the frequency and structure of secondary deformations between boys and girls ($p > 0.05$) were not found.

The comparison of data of epidemiological surveys conducted in 2010 and 2019 showed the increase ($p < 0.05$) of secondary deformations in 2019 and the change of its structure. Thus, in 2010, 40.0 % of secondary deformations were found in surveyed with adentia, and in 2019, 64.1 % were found. At the same time, the percentage of vertical deformations decreased significantly ($p < 0.05$) from 50.0 % in 2010 to 16.0 % in 2019.

Table 3

The frequency of emergence of secondary deformations and the distribution of secondary deformations

Year of research	Gender	Frequency of secondary deformations		
2019	overall (n=279)	64.1 % (n=25)		
	boys (n=137)	33.3 % (n=13)		
	girls (n=142)	30.8 % (n=12)		
	P _{b-g}	>0.05		
2010 [7]	overall (n=150)	40.0 % (n=20)		
P _{ov2019-2010}		<0.001		
Year of research	Gender	Secondary deformations		
		in a vertical plane	in a horizontal plane	mixed
2019	overall (n=25)	16.0 % (n=4)	60.0 % (n=15)	24.0 % (n=6)
	boys (n=13)	23.1 % (n=3)	53.8 % (n=7)	23.1 % (n=3)
	girls (n=12)	8.3 % (n=1)	66.7 % (n=8)	25.0 % (n=3)
	P _{b-g}	>0.05	>0.05	>0.05
2010 [7]	overall (n=20)	50.0 % (n=10)	40.0 % (n=8)	10.0 % (n=2)
P _{ov2019-2010}		<0.05	>0.05	>0.05

The survey revealed a total of 66 missing teeth. Thus, the average intensity of missing teeth per one surveyed with adentia was 1.69, meanwhile for men this indicator was not significantly higher 1.74 than for women 1.65.

The structure of adentia according to separate teeth is shown in table 4.

Table 4

The structure of adentia according to separate teeth

Tooth name	Number of extracted teeth	Percentage of extracted teeth to all missing teeth
18	1	1.5 %
17	1	1.5 %
16	4	6.1 %
15	0	0 %
14	2	3.0 %
13	0	0 %
12	2	3.0 %
11	0	0 %
21	0	0 %
22	2	3.0 %
23	1	1.5 %
24	3	4.5 %
25	2	3.0 %
26	6	9.1 %
27	0	0 %
28	0	0 %
38	0	0 %
37	3	4.5 %
36	12	18.2 %
35	3	4.5 %
34	2	3.0 %
33	0	0 %
32	1	1.5 %
31	0	0 %
41	0	0 %
42	1	1.5 %
43	0	0 %
44	1	1.5 %
45	4	6.1 %
46	12	18.2 %
47	2	3.0 %
48	1	1.5 %
Total	66	

In the structure of missing teeth the significant prevalence of adentia of the posterior group of teeth was indicated. The number of extracted teeth of the anterior group was 10.6 % (n=7) versus 89.4 % (n=59) of extracted teeth of the posterior group.

The distribution of missing tooth on the right and left side wasn't differ much: 53.0 % (n=35) of adentia cases were observed on the left side and 47.0 % (n=31) of adentia cases were observed on the right one.

The adentia of 36.4 % (n=24) of teeth of the maxilla and the adentia of 63.6 % (n=42) of teeth of the mandible were determined. That is, the frequency of adentia emergence on the mandible was two times higher than the one on the maxilla.

Among all adentia cases, more than a half 51.5 % (n=34) were about the first molars. Missing of the second premolars and the first molars accounted for 65.2 % (n=43). Thus, we noted that two thirds of patients were able to potentially receive orthodontic care aimed at the protraction of distal teeth toward the defect.

Changes of dental arches forms in secondary deformations and related formation of supra-occlusal contacts, laterotrusive or mesiotrusive contacts lead to a violation of synchronicity of muscle contraction, work of the elements of temporomandibular joint, removal of masticatory cycles towards the intact side of the jaw, medial, lateral or distal jaw displacement [5]. In addition, there is a decrease of occlusal vertical dimension and the emergence of related orthodontic pathology in cases of adentia [9]. As a result, it causes joint dysfunction and morphological changes in it [14].

The correlation between adentia and degenerative changes in the temporomandibular joint was determined. It is determined that the decrease of molar support as a result of adentia significantly affects the tissues of temporomandibular joint elements, whereas the number of lost teeth is less important [3].

Local changes caused by adentia do not allow providing sufficient prosthodontic care, and require a multidisciplinary approach to the rehabilitation of patients that includes prosthodontic, orthodontic, periodontal care [11].

Conclusions

1. It was found that the frequency of adentia among young people is 14.0 %, with no significant difference between gender groups. The intensity of missing teeth per one surveyed with adentia is 1.69. The comparison of frequency of adentia among young people in 2010 and 2019 revealed an almost 2.5-fold decrease in the incidence of this pathology. The comparison of structure of adentia in 2010 and 2019 revealed a change towards the relief of orthopedic pathology due to the significant decrease of adentia in the form of edentulous areas located posterior to the remaining natural teeth.

2. The comparison of frequency of secondary deformations in 2010 and 2019 revealed their increase in 2019 from 40.0 % to 64.1 % and the change of structure of secondary deformations.

3. In the structure of adentia there is a ninefold prevalence of adentia in the posterior area above the anterior one, a significant prevalence of adentia of the lower jaw. More than a half of adentia cases are those of the first molars (51.5 %). The frequency of adentia of the second premolars and the first molars is 65.2 %.

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Реферати

ЧАСТОТА, СТРУКТУРА ТА ДИНАМІКА РОЗВИТКУ АДЕНТИЙ І ПОВ'ЯЗАНИХ З НИМИ ВТОРИННИХ ДЕФОРМАЦІЙ ЗУБНИХ РЯДІВ У ОСІБ МОЛОДОГО ВІКУ

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В рамках епідеміологічного обстеження стоматологічного здоров'я осіб молодого віку нами проаналізовані дані клінічного стоматологічного обстеження 137 юнаків і 142 дівчат, студентів стоматологічного факультету Вінницького національного медичного університету ім. М. І. Пирогова. Для порівняльного аналізу структури адентій нами були використані дані епідеміологічного дослідження, проведеного у 2010 році на базі кафедри ортопедичної стоматології. Статистичну обробку результатів проведено в ліцензійному пакеті "Statistica 6.0". Порівняння структури та частоти адентій у молодого населення у 2010 та 2019 роках виявило зменшення частоти даної патології. Слід також підкреслити зміну в структурі адентій за топографією в бік полегшення ортопедичної патології внаслідок значного зменшення дистально необмежених форм адентій. Виявлена значна частота вторинних деформацій у обстежених з переважанням горизонтальної форми. У структурі адентій відмічається дев'ятикратне переважання адентій в бічному відділі над фронтальним, значне переважання адентій на нижній щелепі.

Ключові слова: адентії, епідеміологія, вторинні деформації.

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ЧАСТОТА, СТРУКТУРА И ДИНАМИКА РАЗВИТИЯ АДЕНТИЙ И СВЯЗАННЫХ С НИМИ ВТОРИЧНЫХ ДЕФОРМАЦИЙ ЗУБНЫХ РЯДОВ У ЛИЦ МОЛОДОГО ВОЗРАСТА

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Закалата Т.Р.

В рамках эпидемиологического обследования стоматологического здоровья лиц молодого возраста нами проанализированы данные клинического стоматологического обследования 137 юношей и 142 девушек, студентов стоматологического факультета Винницкого национального медицинского университета им. Н. И. Пирогова. Для сравнительного анализа структуры адентий нами были использованы данные эпидемиологического исследования, проведенного в 2010 году на базе кафедры ортопедической стоматологии. Статистическая обработка результатов проведена в лицензионном пакете "Statistica 6.0". Сравнение структуры и частоты адентий у молодого населения в 2010 и 2019 годах выявило уменьшение частоты данной патологии. Следует также подчеркнуть изменения в структуре адентий по топографии в сторону облегчения ортопедической патологии вследствие значительного уменьшения дистально неограниченных форм адентий. Выявлена значительная частота вторичных деформаций у обследованных с превалированием горизонтальной формы. В структуре адентий отмечается девятикратное превалирование адентий в боковом отделе над фронтальным, значительное превалирование адентий на нижней челюсти.

Ключевые слова: адентии, эпидемиология, вторичные деформации.

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PROSPECTS FOR MINERAL METABOLIC DISORDERS CORRECTION IN CHILDREN WITH RECURRENT STATE OF URINARY TRACT INFECTION

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The purpose of the study was to establish the effectiveness of combined phyto-citrate compound in the treatment and prevention of recurrent urinary tract infections in children with saline dysmetabolism in the comparison groups. The study involved 33 children aged 6 to 18 years, patients with recurrent urinary tract infections in the acute exacerbation of the disease. According to the duration of combined phyto-citrate compound administration, children were divided into 2 groups: I (n=17) – received the drug in complex therapy of urinary tract infections for 1 month, II (n=16) – also received phyto-citrate compound in complex therapy for 1 month, but continued to take it to prevent urinary tract infections for the next 2 months in an intermittent mode for 10 days. The results of the study proved phyto-citrate effectiveness in the complex therapy of recurrent urinary tract infections in children on the background of saline dysmetabolism. Administration of this drug led to normalization of saline transport indicators in 82% (27/33) of patients (p<0.05) after the 1st month of treatment and reduced the risk of reinfection by 18 times: OR = 4.25±0.65 with 95% CI [1.18; 15.3] – before treatment and OR = 0.235±0.65 [0.066; 0.846] – after the first month of treatment (p<0.001). Prolonged intermittent administration of the drug for the next 2 months reduced the risk of recurrent episodes of urinary tract infection by 3.5 times: OR = 1.88±0.9 [0.302; 11.73] – in case of choosing a 1-month course of therapy; and OR= 0.53±0.83 [0.085; 3.3], p<0.05 – as a result of preventive use of the combined phyto-citrate.

Key words: recurrent urinary tract infection, children, mineral dysmetabolism, combined phyto-citrate compound.

The work is a fragment of the research project "Study of the hyperuricemia treatment impact in patients with chronic kidney disease and justification of optimal therapy", state registration No. 0119U101718.

A comprehensive study of the factors contributing to the formation of recurrent urinary tract infections (UTIs) in children and analysis of their elimination effectiveness is undoubtedly one of the topical issues of clinical medicine [1].

Dysmetabolic nephropathy (DN) represents from 27% to 64% in the structure of the urinary system incidence in children, and in the daily practice of a pediatrician, the metabolic disorders syndrome in the

urine is observed in almost every third patient [6]. Some foreign studies also emphasize the increase in the incidence of recurrent UTIs combined with crystalluria [4]. Antibiotics can increase the risk of developing renal calculi, as found by American scientists [11]. Studies have shown that antibiotics can change the intestinal microflora by reducing the colonization of oxalate-degrading bacteria *Oxalobacter formigenes* [10]. This makes patients sensitive to the renal calculi formation. It is concluded that taking antibacterial drugs significantly reduces the frequency of *Oxalobacter formigenes* colonization, and the negative impact persists for at least 6 months [3].

Therefore, given the latest data, disorders of mineral metabolism are considered not only as a concomitant urological condition that contributes to the development and maintenance of urinary tract infections, but also as one that can be initiated and maintained by the infectious-inflammatory process in the urological tract [5, 9].

The purpose of our study was to establish the effectiveness of mineral dysmetabolism correction in children with recurrent UTIs and its impact on the course of the disease by including phyto-citrate compound in the complex therapy.

Materials and methods. During 2019, a randomized controlled clinical trial was carried out. 33 children aged 6 to 18 years with recurrent UTIs in combination with impaired mineral metabolism participated. The study was performed with the informed consent of parents, children and in accordance with the Declaration of Helsinki on Human Rights.

The study design of patients was observed in 2 comparison groups. In Group I ($n = 17$), children received basic standard treatment (antibiotic, antispasmodic drug, antipyretic agent, if required) enhanced by phyto-citrate (PC) compound (sodium citrate + potassium citrate + phytocomponents: powdered pericarp of French bean (*Phaseolus vulgaris*), dry extracts of Asian white birch (*Betula platyphylla*) leaves, Garden parsley (*Petroselinum crispum*) root, lingonberry (*Vaccinium vitis-idaea*) leaves, German chamomile (*Matricaria chamomilla*).

The duration of PC treatment at a dose of 1 capsule 2 times a day for children aged 6 to 11 years and 1 capsule 3 times a day for children aged 12 to 18 years for 1 month. In the second study group ($n = 16$), children also received PC as part of complex therapy in appropriate age-specific doses (as indicated above). The difference was in the PC term and administration schedule. The total duration of PC administration in this study group was 3 months. During the first month, administration schedule of PC was similar to Group I, and in the next 2 months – 10-day administration of the drug alternated with a 10-day break (according to the recommendations of some authors in the case of prolonged courses of administration) under conditions of stable urine pH in the range of 6.2–6.8.

Patients were randomized using the STATISTICA application program package of the random number generator (even numbers corresponded to one group, odd numbers to another). Nonparametric statistical methods were used for analytical and mathematical processing. During testing of statistical hypotheses, the threshold value for the significance level was set at $p \leq 0.05$ [2].

Mathematical processing of the obtained data was performed using Microsoft Excel software (Microsoft Office 2013 Professional Plus, license agreement (EULAID: O15_RTM_VL.1_RTM_RU) and STATISTICA 13.0 (StatSoftInc., serial No. ZZS9990000099100363DEMO-L).

Results of the study and their discussion. The study involved children aged 6 to 18 years, the mean age was 10.8 ± 4.2 . Among them were 76% girls (25/33) and 24% (8/33) boys. All patients at the time of involvement in the study had a clinical and laboratory manifestation of the disease. According to the nosology, there were 55% (18/33) of patients with chronic cystitis, with chronic pyelonephritis – 45% (15/33). The distribution of patients by age, sex, diagnosis and clinical and laboratory characteristics of the disease in the comparison groups is shown in table 1.

Therefore, patients were randomized, and no statistically significant discrepancy in these parameters in the comparison groups was found ($p > 0.05$).

Among this group, crystalluria was present in the vast majority of children in the form of oxaluria in 60% (20/33), urate accumulations occurred in 19% (6/33), a combination of urate accumulations with phosphaturia, or oxaluria in 21% (7/33) of patients.

Urinary response in most patients in both study groups at the beginning of treatment was alkaline, only in 19% (6/33) patients with persistent urate accumulations an acidic urine pH was determined. At the end of the 4th week of therapy, all patients had a stable fluctuation of urine pH in the range of 6.5–7.0. This fact was interpreted by us as an indirect sign of an increase in the level of urine citrate, which indicated an improvement in the lithogenic properties of urine. It is known that citrate is a dissociated anion of citric acid, an energy substrate of the TCA cycle that has a pronounced effect on purine metabolism [8]. It also has a direct inhibitory effect on the crystallization and precipitation of calcium salts, that is, it is an inhibitor

of renal calculi formation. And being one of the most important natural mechanisms of crystallization inhibition, citrate excretion in the urine depends on the state of acid-base homeostasis [14].

Table 1

Distribution of patients by main characteristics in comparison groups

Parameters		Group I, n=17	Group II, n=16	p
Age		10.3±5.2	10.9±3.6	p=0.88
Sex	Female	13/17	12/16	p=0.68
	Male	4/17	4/16	p=0.68
Body temperature °C,		37.4±0.27	37.5±0.63	p=0.48
Dysuria, n		13/17	14/16	p=0.84
Pain syndrome, n		9/17	7/16	p=0.72
Periorbital edema		8/17	8/16	p=0.84
Crystalluria		9/17	8/16	p=0.94
Bacteriuria		14/17	12/16	p=0.62
Leukocytosis, 10 ⁹ /l		5.2±1.4	5.6±1.23	p=0.86
CRP, mg/l		22.43±3.46	21±6.7	p=0.62
Leukocyturia, HPF		29.6±11.8	26±19.9	p=0.42
Haematuria, HPF		18.92±0.35	20.2±0.26	p=0.72
Proteinuria, g/per day		0.12±0.06	0.18±0.07	p=0.42
Accumulation of salts on the ultrasound		10/17	11/16	p=0.65
CP		7/17	8/16	p=0.79
CC		10/17	8/16	p=0.45

Notes: CP – chronic pyelonephritis, CC – chronic cystitis. p>0.05 – an incredible indices discrepancy between the χ^2 criterion and the Yates's correction.

Leveling of clinical and laboratory signs of infectious and inflammatory process was detected at the end of 2 weeks of therapy in most (82%) of patients in both study groups (14/17 and 13/16, respectively). In them there was not found any dysuria, pain syndrome, normalization of body temperature (in all children), diuresis, CRP levels, leukocyturia, leukocytosis (table 2).

Table 2

Dynamics of clinical and laboratory signs of UTIs at the end of the 2nd week of treatment

Parameters	Group I, (n=17)		Group II, (n=16)		p
	before treatment, abs.	after 2 weeks of treatment	before treatment, abs.	after 2 weeks of treatment	
Clinical characteristics					
Abdominal/lower back pain	9/17	2/17	7/16	1/16	p=0.79
Dysuria	13/17	3/17	14/16	3/16	p=0.89
Periorbital edema in the morning	8/17	4/17	6/16	4/16	p=0.89
Loss of appetite	12/17	4/17	10/16	3/16	p=0.89
Sediment in a fresh portion of urine	10/17	15/17	9/16	14/16	p=0.79
Laboratory and instrumental data					
Urine output, l/day	1.0±0.04	1.4±0.03	0.9±0.04	1.2±0.04	p=0.79
urine pH	7.28±0.06	6.52±0.04	7.02±0.05	5.58±0.06	p=0.69
White blood cells, HPF	25.31±0.4	5.67±0.6	24.89±0.4	6.87±0.7	p=0.79
Red blood cells, HPF	18.92±0.35	4.32±0.3	16.85±0.41	5.84±0.4	p=0.79
Protein, g/per day	0.12±0.06	0.034±0.04	0.091±0.05	0.062±0.06	p=0.46
Bacteriuria	14/17	5/17	14/16	3/16	p=0.64
Accumulation of salts on the ultrasound	9/17	4/17	9/16	5/16	p=0.79

Note. p>0.05 – an incredible indices discrepancy between the χ^2 criterion and the Yates's correction after 2 weeks of treatment in experimental groups.

Increased saluresis at the 2nd week of follow-up in an average of 88% (in 15 of 17 patients in Group I and in 14 of 16 patients in Group II) was interpreted as a positive crystalline and lithokinetic effect of complex phyto-citrate therapy. Moreover, due to the antispasmodic and anti-inflammatory action of PC, litholysis was not accompanied by increased dysuria, the presence of pain and did not lead to urinary tract obstruction.

Average values of mineral metabolism according to the results of saline transport corresponded to the norm in 82% of patients (14/17 and 13/16), p<0.05 at the end of the 1st month of therapy (fig. 1). Regression of the urinary syndrome in the form of a decrease in the levels of proteinuria and haematuria to the "micro" level became probable also after the 1st month of therapy (fig. 2).

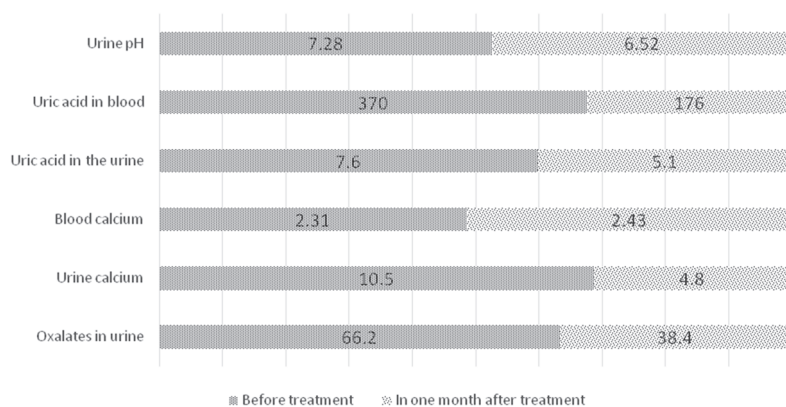


Fig. 1. Dynamics of changes in mineral metabolism in terms of saline transport at the end of the 1st month of therapy in Group I.

Note. $p > 0.05$ – an incredible indices discrepancy between the χ^2 criterion and the Yates's correction in comparison with Group II.

tion; affects the metabolism of purines in the organism by blocking aminogenesis, causes a decrease in the content of ammonia and purine derivatives in blood plasma, which contributes to hypouricemia [12].

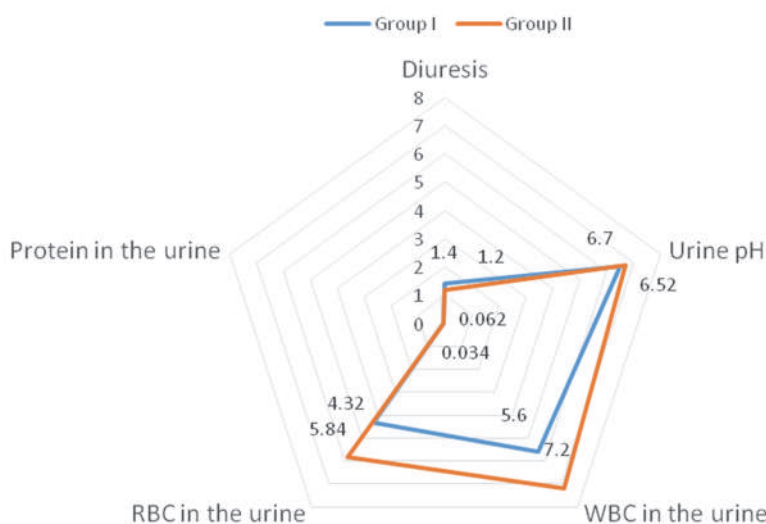


Fig. 2. Comparative dynamics of regression of urinary syndrome at the end of the 1st month of therapy in experimental groups

Note. $p > 0.05$ – an incredible indices discrepancy between the χ^2 criterion and the Yates's correction between experimental groups.

the action of phyto-citrate compound. This effect may have been provided by the action of birch leaf extract (*Betula platyphylla*), which is able to reduce albuminuria by improving microcirculation in the renal parenchyma and regulation of reabsorption in the tubules.

Patients in both groups at the end of the one month-long course of therapy remained comparable in terms of basic clinical and laboratory parameters. Analysis of the odds ratio (OR) showed an 18-fold higher probability ($p < 0.001$) of the UTIs development before complex treatment (antibacterial therapy + complex phyto-citrate) in Groups I and II. Thus, the OR index before therapy was $OR = 4.25 \pm 0.65$ at 95% CI [1.18; 15.3], after therapy- $OR = 0.235 \pm 0.65$ [0.066; 0.846].

Subsequently, for 2 months, group I underwent clinical follow-up for repeated episodes of UTIs without medication. The second group of patients continued therapy with PC complex, when 10-day administration of the drug alternated with a 10-day break for the next 2 months.

According to the literature analysis, the minimum course of citrate therapy can be carried out for 1-2 months, extended to at least 6 months, with cystine formations – even up to 12 months. A number of scientists believe that to maintain normal values of mineral metabolism, constant administration of citrate mixtures is not required. Instead, after a stable establishment of favorable trends in urine chemistry (urine pH at the level of 6.2– 6.8), it is possible to perform 10-day administration of the drug alternated with a 10-day break for the required period [13].

As a result of a further 2-month follow-up of patients, repeated episodes of UTIs were registered in 4 patients of Group I (23.5%) who did not receive prophylactic therapy, and in 2 patients of Group II (12.5%) who received complex phyto-citrate compound as prophylaxis. Therefore, the probability of

As can be seen from fig. 1, at the end of the 1st month of phyto-citrate therapy, there was a relevant decrease in patients of the mean values of oxalates excretion in the urine by 1.7 times, hyperuricemia level by 2.1 times, hyperuricosuria by 1.5 times, urinary calcium excretion by 2.2 times ($p < 0.05$). The obtained results fully correspond to modern ideas about the proven effect of citrate alkalization, which increases the dissociation of uric acid salts and reduces the tendency to renal calculi formation.

Haematuria, crystalluria, salt inclusions according to ultrasound examination had the most likely regression at the end of the 1st month of therapy, which probably contributed to the capillary-protective effect of lingonberry leaf extract (*Vaccinium vitis-idaea*) lingonberry and pericarp of beans (*Phaseolus vulgaris*) in combination with the litholytic action of parsley root extract (*Petroselinum crispum*) and the crystallic-disintegrating action of potassium – sodium citrate component. An interesting fact was the regression of microproteinuria ($p < 0.05$) due to

recurrence in the case of choosing the therapeutic approach of Group I was $OR=1.88\pm 0.9$ [0,302; 11,73] at 95% CI, whereas in the case of choosing the therapy used in the second study group – $OR = 0.53\pm 0.83$ [0.085; 3.3], ($p < 0.05$). That is, the risk of UTIs recurrence was 3.5 times lower if you adhere to prophylactic therapy with PC for the next 2 months after completion of the basic 1 month-long course of UTIs treatment.

The obtained results coincide with the literature data, which indicate the pathogenetic contribution of impaired mineral metabolism metabolites in maintaining the recurrent course of UTIs and indicate the relevance of its correction in order to increase the treatment effectiveness and disease prevention [7]. According to many authors, therapeutic measures in the combination of UTIs and mineral dysmetabolism should be aimed both at the eradication of microorganisms, and litholysis of calculi elimination [15]. A number of scientists have concluded that it is drugs with a combination of sodium citrate and potassium citrate provide colloidal crystals stability of urine in different types of kidney stone diseases. Since 2011, according to the recommendations of the European Association of Urologists, citrate drugs have become a mandatory component of therapy for patients with urolithiasis and other hypocitrate conditions [7, 12].

Conclusions

1. The complex of therapy (antibiotic + complex phyto-citrate) in children with recurrent UTIs in the phase of infectious exacerbation was effective and reduced the risk of reinfection by 18 times ($p < 0.001$).
2. The inclusion of phyto-citrate in the treatment regimen of patients with combined UTIs and mineral dysmetabolism led to normalization of salt transport in 82% (27/33), $p < 0.05$ patients at the end of the 1st month of therapy.
3. Prophylactic administration of the complex phyto-citrate compound (in the regimen of alternating 10-day administration and 10-day break) for 2 months after the course of UTIs treatment, reduced the risk of recurrence by 3.5 times, $p < 0.05$.

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Реферат

**ПЕРСПЕКТИВИ КОРЕКЦІЇ
МІНЕРАЛЬНОГО ДИЗМЕТАБОЛІЗМУ
В ДІТЕЙ ІЗ РЕКУРЕНТНИМ ПЕРЕБІГОМ
ІНФЕКЦІЇ СЕЧОВОЇ СИСТЕМИ**

Буднік Т.В., Квашніна Л.В.

Метою дослідження було вивчення ефективності застосування комбінованого фітоцитратного засобу в комплексній терапії та профілактиці рекурентної інфекції

**ПЕРСПЕКТИВЫ КОРРЕКЦИИ
МИНЕРАЛЬНОГО ДИЗМЕТАБОЛИЗМА
У ДЕТЕЙ С РЕКУРРЕНТНЫМ ТЕЧЕНИЕМ
ИНФЕКЦИИ МОЧЕВОЙ СИСТЕМЫ**

Будник Т.В., Квашнина Л.В.

Целью исследования было изучение эффективности комбинированного фиточитратного средства в комплексной терапии и профилактике

сечової системи у дітей на тлі сольового дизметаболізму в групах порівняння. В дослідженні прийняли участь 33 дитини віком від 6 до 18 рр., хворі на рекурентну інфекцію сечової системи в стадію загострення. За тривалістю застосування комбінованого фітоцитрату дітей було поділено на 2 групи: I (n=17) – отримували засіб в комплексній терапії ІСС протягом 1 місяця, II (n=16) – також отримували фітоцитратний засіб в комплексній терапії протягом 1 місяця, але продовжили його прийом у цілях профілактики ІСС ще наступні 2 місяці у переривчастому режимі по 10 днів.

Результати дослідження довели ефективність застосування комбінованого фітоцитрату у комплексній терапії рекурентної ІСС у дітей на тлі сольового дизметаболізму. Застосування зазначеного засобу призводило до нормалізації показників транспорту солей у 82 % (27/33) пацієнтів (p<0,05) вже після 1-го місяця терапії й знижувало ризик реінфекції в 18 разів: OR = 4,25 ± 0,65 при 95% ДІ [1,18; 15,3] – до терапії та OR = 0,235 ± 0,65 [0,066; 0,846] - після першого місяця терапії, (p<0,001). Пролонгований переривчастий прийом препарату протягом наступних 2-х місяців зменшував ризик повторних епізодів інфекції сечової системи в 3,5 рази: OR = 1,88 ± 0,9 [0,302; 11,73] - у разі вибору 1-місячного курсу терапії; та OR = 0,53 ± 0,83 [0,085; 3,3], p<0,05 – у результаті профілактичного застосування комбінованого фітоцитрату.

Ключові слова: рекурентна інфекція сечової системи, діти, мінеральний дизметаболізм, комбінований фітоцитратний комплекс.

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рекурентної інфекції мочевої системи у дітей на фоні сольового дизметаболізму в групах порівняння. В дослідженні прийняли участь 33 ребенка в візасте от 6 до 18 лет с рекуррентной инфекцией мочевої системы в стадию обострения. Пациенты I группы (n = 17) – получали комбинированный фитощитрат в комплексной терапии в течение 1 месяца, II группы (n = 16) – также получали фитощитратное средство с целью терапии в течение 1 месяца, но продолжили его прием в целях профилактики повторного эпизода еще 2 месяца в прерывистом режиме по 10 дней.

Применение указанного средства приводило к нормализации показателей транспорта солей в 82% (27/33) пациентов (p < 0,05) уже после 1-го месяца терапии и снижало риск реинфекции в 18 раз: OR = 4,25 ± 0,65 при 95% ДІ [1,18; 15,3] – до терапии и OR = 0,235 ± 0,65 [0,066; 0,846] - после первого месяца терапии (p<0,001). Пролонгированный прерывистый прием препарата в течение следующих 2-х месяцев уменьшал риск повторных эпизодов в 3,5 раза: OR = 1,88 ± 0,9 [0,302; 11,73] - в случае выбора 1-месячного курса терапии и OR = 0,53 ± 0,83 [0,085; 3,3], p < 0,05 - в результате профилактического применения комбинированного фитощитрата.

Ключевые слова: рекуррентная инфекция мочевої системы, дети, минеральный дизметаболизм, комбинированный фитощитратный комплекс.

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CHOICE OF TERMS FOR SURGICAL TREATMENT OF UNSTABLE PELVIC RING IN VICTIMS WITH POLYTRAUMA BASED ON ANATOMY-FUNCTIONAL ASSESSMENT OF THE TRAUMA SEVERITY

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The purpose of the work was to improve the results in patients with unstable pelvic injuries in polytrauma. The study of integral rheography indices of the body was performed and some laboratory blood parameters in 137 patients with unstable pelvic injuries in polytrauma (UPIP) were studied. The victims had unstable pelvic injuries, namely types B and C according to the international classification of AO (M. Tile, 1995). Rotationally unstable fractures (type B) were observed in 101 (73.72%) cases, vertically unstable (type C) - in 36 (26.28%). It has been scientifically substantiated that the severity of anatomical injuries and the prediction of the condition for patients with UPIP upon admission should be more informatively assessed using the ATS scale (statistical significance - 89.29%). Surgical interventions on the pelvic bones in case of mild and severe trauma are possible from the 5th-7th days of traumatic disease, with extremely severe trauma - from 10-14 days of TC with a favorable prognosis, which was determined taking into account the indices of integral body rheography.

Keywords: polytrauma, unstable pelvic injuries, integral rheography.

The work is a fragment of the research project "Розробити систему оцінки тяжкості бойової хірургічної травми. To develop a system for assessing the severity of combat surgical trauma", state registration No. 0116U007313.

The high percentage of mortality (up to 70%), complications (up to 80%), disability (up to 68%) permits to consider the problem of treatment in victims with unstable pelvic injuries with polytrauma to be one of the most relevant in modern traumatology and surgery [5].

Tactics of treating victims with unstable pelvic injuries in polytrauma should be differentiated and depend on the injury severity and the prognosis of the traumatic disease (TD) clinical course, the nature of the combined injury, TD period, type of post-traumatic pelvic ring instability, nature of pelvic injuries [1, 6]. The injury rate of traditional surgical methods of treatment in pelvic bone injuries, the severity of other anatomical areas' injuries, the combined trauma of the pelvic organs encourages to avoid early restoration

of the pelvic ring integrity. The main purpose is to save the patient's life, then - functional results, which are better than in the earlier adequate surgical correction of the unstable pelvic ring performed since the moment of injury [8, 9].

And if for the acute period of TD (1 - 2 days) there are established indications and methods of surgical hemostasis and pelvic stabilization [9, 10], then for the early (3 - 7 days) and late (8 - 21 days) TD periods, at present time, there are no clear criteria for the objective choice of surgery terms due to unstable pelvic injuries, the severity of the injury, the nature of the damage to other anatomical areas and pelvic organs.

The purpose of the work was to improve the results of treating victims with unstable pelvic injuries in polytrauma based on the use of the developed anatomical and functional scale for assessing the severity of injury to select the optimal timing of surgical treatment.

Materials and methods. A study of integrated body rheography (IBR) and some laboratory blood parameters was performed in 137 victims with unstable pelvic injuries with polytrauma (UPIP), who were hospitalized at the Kyiv City Clinical Emergency Hospital from 2000 to 2014. Among them, there were 82 survived patients (59.85%), 61 dead ones (40.15%). The mean age of patients was 42.4 ± 2.58 years.

According to the mechanism of injury, 54 (39.4%) patients had catatrauma, 30 (21.9%) - compression with distraction, 28 (20.4%) - direct stroke, 25 (18.3%) - combination of mechanisms. Pelvic trauma and damage to another anatomical area (AA) was observed in 14 (10.21%) patients, another 2 AA - in 48 (35.04%) patients, another 3 AA - in 49 (35.77%) patients, 4 and more AA - in 26 (18.98%) victims. All victims had unstable pelvic injuries, namely types B and C according to the AO international classification. Rotational-unstable fractures (type B) were observed in 101 (73.72%) cases, vertically-unstable (type C) - in 36 (26.28%) cases.

The severity of anatomical injuries was assessed according to the ATS scale, developed by the staff of the Department of Military Surgery at Ukrainian Military Medical Academy (UMMA) [3, 5, 6]. According to this scale, it was found that minor injury ($ATS \leq 24$ points) occurred in 43 (31.38%) victims, severe injury ($ATS - 25-41$ points) - in 57 (41.61%), extremely severe injury ($ATS \geq 42$ points) - in 37 (27.01%). Severe and extremely severe head injuries were observed in 83 (60.58%) cases, those of chest - in 25 (18.25%), abdomen - in 20 (14.60%), skeletal bones and spine - in 86 (62.77%). In 37 (27.01%) cases, unstable pelvic injuries were combined with pelvic organs' injuries.

All victims were admitted in a state of traumatic shock of varying severity. However, it can be noted that the II-III degree traumatic shock prevailed, respectively in 126 (91.97%) cases; at the same time, the I degree shock was only observed in 7 (5.11%), patients and the terminal condition (the IV degree shock) only - in 4 (2.92%) cases (all victims died).

Functional changes in central hemodynamics and respiration were recorded using IBR which was performed according to the method of M.I. Tishchenko [2] using the device KSVG-1 and "Diamond" on days 1-3, 5-7, 10-14, 15-21 after injury, at the same time laboratory blood counts were determined by conventional methods. Anatomical and functional assessment of the injury severity in different periods of traumatic disease was performed according to our own developed scales ATS and AFI [3, 4].

Results of the study and their discussion. In order to monitor the clinical course of trauma and the choice of differentiated surgical tactics for treatment of victims with polytrauma, the Military Surgery Department of UMMA developed a method for anatomical and functional assessment of trauma based on IBR by M.I. Tishchenko, which was improved in the dissertation of O.I. Zhovtonozhko [3, 4].

The calculation of anatomical and functional assessment of injuries and multifactorial assessment of the condition severity with the prognosis of the traumatic disease course and the development of fatalities were assessed by the formulas:

1. Anatomical and functional index:

$$AFI = ATS - 0.13 \times BN + 302.9 - 2.75 \times SVI + 5.2 \times BSI + VTSI + 2.43 \times ITF$$

1. Multifactorial index:

$$0.15 \times SVI + 0.45 \times BSI + 0.117 \times ITF + 0.0647 \times VTSI + 2.33 \times BN - 37$$

$$MFI = \frac{\text{AFI} - \text{AFI}_{\text{min}}}{\text{AFI}_{\text{max}} - \text{AFI}_{\text{min}}} \times 37.3$$

AFI - anatomical and functional index, MFI - multifactorial index, ATS - trauma anatomic assessment scale, IBR indices: BSI - breathing strain index, VTSI - vascular tone stabilization index, ITF - integrated tonic factor, SVI - stroke volume index, BN - percentage of banded neutrophils.

The prognosis of consequences for life was determined taking into account AFI and MFI with allocation of 3 clinical and prognostic groups: with favorable, doubtful and unfavorable consequences for

life. The diagnostic accuracy was 90.75% (for a favorable prognosis - 95.45%, for unfavorable - 86.05%) (table 1).

Table 1

Distribution of victims with UPIP according to the traumatic disease prognosis

Prognosis	Anatomical and functional index – AFI (points)	Multifactorial index – MFI ((points)	Injured (n=137)	
			Survived	Died
favorable	less than 600	less than 0.8	42	2
doubtful	more than 600 less than 600	less than 0.8 more than 0.8	28	22
unfavorable	more than 600	more than 0.8	6	37

In patients with UPIP and a favorable TD prognosis (n = 44) the severity of respiratory and circulatory disorders on days 1–3 after admission was determined as moderately reduced (SVI = 33.9±4.7 ml / m², cardiac index (CI) = 2.9±0.1 l / min / m²). Cardiac output was restored for 5–7 days (SVI=37.9±2.8 ml / m²; CI = 3.1±0.03 l / min / m²), and up to 10–14 days - corresponded to the normodynamic regime of systemic circulation (SVI = 44.9±1.1 ml / m², CI = 3.3±0.02 l / min / m²). The expressed respiratory failure which was observed for 1–3 days after receipt (BSI = 36.7±6.4 RU.; reserve index (RI) = 0.95±0.05 units) to 10–14 days was eliminated (BSI). = 24.2±.8 units; RI = 1.02±0.04 units). Moderate centralization of blood circulation (ITF = 71.5±0.2 RU) was maintained until the end of the acute period of traumatic disease and was eliminated on days 5-7 (ITF = 75.2±0.2 RU) during TD (table 2).

Table 2

Dynamics of changes in respiratory and circulatory disorders in UPIP patients with favorable prognosis of TD (mild trauma)

IBR index	Days 1–3 (n=44)	Days 5–7 (n=44)	Days 10–14 (n=44)
SVI, ml/m ²	33.9±4.7	37.9±2.8*	44.9±1.1*
CI, l/min/m ²	2.9±0.1	3.1±0.03*	3.3±0.02*
BSI, RU	36.7±6.4	33.4±5.7	24.2±1.8
VTSI, RU	1.4±0.1	1.2±0.1	1.02±0.1
RI, RU	0.95±0.05	0.99±0.04	1.02±0.04
ITF, RU	71.5±0.2*	75.2±0.2*	75.7±0.1*

Note: * - the difference is statistically significant (p < 0.05).

Post-traumatic indolent anemia was observed at the end of the first day after admission (hemoglobin = 106.2±2.63 g / l, erythrocytes = 3.4±0.06x10¹² / l) and persisted for up to 3 days (hemoglobin = 104.5±4.12 g / l, erythrocytes = 3.5±0.05x10¹² / l), (p < 0.05). The hematocrit number was the same: 0.31±0.02. Decrease in the number of erythrocytes in traumatic disease was due not only to the blood loss, but also to their increased consumption due to hyperaggregation and destruction.

In the acute period of TD, from the first hours, neutrophilic leukocytosis (leukocytes = 10.8±0.75x10⁹ / l, rod-shaped neutrophils = 6.52±0.72%) was observed.

The erythrocyte sedimentation rate after 24 hours remained high (11.07±0.79 mm / h and 13.16±0.81 mm / h, respectively), and the level of albumin on days 1 and 3 was consistently low (51.8±1.54% and 48.3±1.22%, respectively). On the first day, the patients developed moderate hypercoagulation (fibrinogen = 4.96±0.32 g / l), which was observed after the acute period of traumatic disease (fibrinogen = 4.21±0.18 g / l) against the background of normal fibrinolytic blood activity.

In patients with UPIP and mild trauma, who survived (n = 42), the prognosis of TD was determined throughout treatment as favorable: by AFP from 587.4 to 550.7 points and by BFP from 0.78 to 0.69 points.

In 28 victims with severe combined pelvic injury (520–620 by AFP), who survived, a questionable prognosis was obtained on admission (by ATS scale). By days 5–7, the prognosis in all victims became favorable (by AFP and BFP). The cardiac output was restored up to days 5–7 (SVI – 48.21±1.27 ml / m² compared to days 1–3: SVI – 40.12±0.95 ml / m², p < 0.05), and up to days 10–14 - corresponded to the hyperdynamic regime of systemic circulation (SVI – 60.12±2.05 ml / m², CI – 3.97±0.14 l / min / m²; p < 0.05 compared to the level at admission). Moderate respiratory failure, which was observed on days 1–3 after admission (BSI – 26.93±0.21 RU, RI – 1.24±0.07 RU) was eliminated by days 15–21, p < 0.05). Moderate centralization of blood circulation (ITF – 78.01±0.35 RU) was maintained until the 15th – 21st day (ITF– 77.6±0.2 RU; p > 0.05) (table. 3).

In victims who died (n = 22), a questionable prognosis (by the ATS scale) was also obtained on admission. At the end of the 3rd day and in other periods, the prognosis was consistently unfavorable (by AFP and BFP). There was a critical decrease in IBR indices in the acute period of TD: SVI to 17.8±3.1 ml

/ m²; CI to 1.9±0.3 l / min / m², ITF to 63.8±0.4 RU, RI to 0.43±0.05 RU. And critical increase of VTSI to 1.92±0.04 RU and BSI up to 62.8±8.3 RU. In the following periods of TD, the IBR indices remained virtually unchanged and ranged from critical to severe reductions, which indicated an extremely severe condition of the victims and an unfavorable prognosis (table 3).

Table 3

Dynamics of changes in respiratory and circulatory disorders in patients with UPIP (severe trauma)

Trauma consequence	IBR index	Day			
		1-3	5-7	10-14	15-21
Survived n=28	SVI, (ml/m ²)	40.12±0.95	48.21±1.27	60.12±2.05	45.2±1.2
	CI, (l/min / m ²)	2.31±0.25	3.12±0.52	3.97±0.14	3.2±0.2
	ITF, (RU)	79.45±0.81	79.01±0.55	77.98±0.32	77.6±0.2
	VTSI, (RU)	1.52±0.03	1.35±0.02	1.2±0.01	1.09±0.01
	BSI, (RU)	26.93±0.21	25.1±0.40	24.32±1.85	25.3±0.23
	RI, (RU)	1.24±0.07	1.27±0.14	1.42±0.07	1.06±0.13
Died n=22	SVI, (ml/m ²)	17.8±3.1*	19.4±2.6*	23.6±2.1*	21.8±1.8*
	CI (l/min/m ²)	1.49±0.3*	1.64±0.2*	2.12±0.3*	1.7±0.1*
	ITF, (RU)	63.8±0.4*	68.2±0.3*	71.6±0.5*	73.1±0.2*
	VTSI, (RU)	1.92±0.04*	1.69±0.02*	1.3±0.02*	1.16±0.01*
	BSI, (RU)	62.8±8.3*	69.5±7.3*	74.1±6.3*	72.9±5.9*
	RI, (RU)	0.43±0.05*	0.55±0.04*	0.6±0.04*	0.65±0.03*

Note: * - the difference is statistically significant compared to the survived victims (p < 0.05).

In the analysis of laboratory parameters in victims who survived, moderate anemia persisted up to days 10-14 (Hb - 100.25±3.12 g / l). A slight increase in the concentration of leukocytes and banded neutrophils was observed on days 1-3 after injury (8.24±0.85x10⁹ / l, 7.64±1.94%, respectively) with a significant decrease up to days 5-7 (p < 0.05). Noteworthy is the decrease in the concentration of blood albumin up to days 10-14 (36.98±1.62 g / l) with a moderate increase to day 21 after injury (49.25±1.27 g / l) (p < 0.05). No significant changes in the blood coagulation system were observed (fibrinogen concentration ranged from 3.82±0.15 g / l on days 1-3 up to 4.76±0.21 g / l on days 10-14 (p < 0.05).

In 6 victims with extremely severe combined pelvic injury (by AFI more than 620 points), who survived, an unfavorable prognosis was obtained on admission (by the ATS scale). Up to days 10-14, the prognosis in all victims became favorable (by AFI and MFI). Upon admission, there were pronounced respiratory and circulatory disorders (a sharp decrease in myocardial contractility: SVI-27.05±1.07 ml / m², CI - 1.82±0.12 l / min / m², moderate stress of the external respiratory system - BSI - 32.74 ± 0.63 RU) with the subsequent normalization of cardiac activity up to day 10 (SVI - 51.06±3.21 ml / m², CI - 3.41±0.12 l / min / m²), p < 0.05), and external respiratory systems - up to days 15-21 (BSI - 24.5±1.19 RU, p < 0.05) (table 4).

Table 4

Dynamics of changes in respiratory and circulatory disorders in UPIP victims (extremely severe injury)

Trauma consequence	IBR index	Day			
		1-3	5-7	10-14	15-21
survived n=6	SVI, (мл/м ²)	27.05±1.07	34.76±1.18	51.06±3.21	46.4±1.3
	CI, (л/хв/м ²)	1.82±0.12	1.92±0.05	3.41±0.12	3.1±0.02
	ITF, (ум.од.)	76.15±0.23	78.01±0.35	79.14±0.78	76.9±0.2
	VTSI, (ум.од.)	1.46±0.03	1.39±0.02	1.15±0.01	1.03±0.01
	BSI, (ум.од.)	32.74±0.63	28.12±0.28	27.03±0.38	24.5±1.19
	RI, (від.од.)	1.14±0.25	1.42±0.08	1.44±0.07	1.01±0.01
died n=37	SVI, (мл/м ²)	24.14±1.15	23.21±1.62*	15.10±0.27*	-
	CI, (л/хв/м ²)	1.84±0.35	1.82±0.14	1.74±0.13*	-
	ITF, (ум.од.)	74.15±2.18	72.16±1.72*	71.32±0.24*	-
	VTSI, (ум.од.)	2.01±0.06*	2.06±0.09*	2.18±0.07*	-
	BSI, (ум.од.)	42.18±4.27*	54.14±5.18*	73.21±8.27*	-
	RI, (від.од.)	0.96±0.10	0.92±0.17*	0.57±0.02*	-

Note: * - the difference is statistically significant compared to the survived victims (p < 0.05).

In victims with extremely severe combined pelvic trauma who died (n = 37), an unfavorable prognosis was determined (according to the ATS scale) on admission. Subsequently, on days 5-7, there was an increase in the functional component of the trauma severity (AFI-836.7±13.2 points, MFI-1.78±0.25), followed by decompensation on days 10-14 (AFI -965.9±15.4 points, MFI-2.35±0.12), p < 0.05. In the analysis of respiratory and circulatory disorders in this group of victims, a sharp decrease in myocardial contractility, starting from days 5-7 and until death (days 10-14) (SVI-23.21±1.62 ml / m²),

SVI – 15.10 ± 0.27 ml / m², respectively, $p < 0.05$). Respiratory failure against the background of the external respiratory system's strain was indicated by the BSI increase on days 5-7 compared to days 1-3 (from 42.18 ± 4.27 RU to 54.14 ± 5.18 RU, $p < 0.05$), and on days 10–14 this index increased almost by 2 times (BSI – 73.21 ± 8.27), $p < 0.05$. The general blood circulation failure was characterized by decrease in the reserve index (RI) from days 5–7 to days 10–14 almost by 1.5 times (RI – 0.92 ± 0.17 RU, 0.7 ± 0.02 RU) respectively, $p < 0.05$ (table. 4).

In victims with extremely severe pelvic trauma, who survived, it was found that anemia was observed in all periods of TD and was more significant up to days 5-7 (hemoglobin – 87.95 ± 1.17 g / l; $p < 0.05$ compared to the level on days 1–3). From the first hours after the injury leukocytosis was reported, which was observed up to day 14 (on days 1-3 - $11.26 \pm 0.15 \times 10^9$ / l, on days 10-14 - $9.27 \pm 0.54 \times 10^9$ / l) with a significant decrease on days 15–21 - $8.77 \pm 0.42 \times 10^9$ / l, $p < 0.05$). The number of rod nuclear leukocytes decreased up to days 5–7 (banded neutrophils – $10.31 \pm 1.05\%$), and on days 10–14 - normalized: banded neutrophils - $5.21 \pm 0.04\%$ ($p < 0.05$). In all periods of TD, the erythrocyte sedimentation rate increased almost by 2 times. The amount of total protein and its fraction - albumin normalized up to days 15- 21 (on days 10-14 - 33.13 ± 1.45 g / l, on days 15-21 - 45.56 ± 1.25 g / l, $p < 0.05$). Moderate hypercoagulation was observed starting from days 5–7 (fibrinogen – 5.49 ± 0.52 g / l) with a slight increase up to days 10–14 (fibrinogen – 5.49 ± 0.52 g / l), $p > 0.05$ and a significant decrease up to days 15–21 (fibrinogen – 5.14 ± 0.41 g / l), $p < 0.05$.

Among the victims who died, there was a progressive decrease in hemoglobin concentration starting from days 5-7 to death compared to the survived group ($p < 0.05$), increasing leukocytosis (days 1-3 - $11.93 \pm 0.76 \times 10^9$ / l, on days 10–14 - $14.22 \pm 0.17 \times 10^9$ / l, $p < 0.05$), with an increase of more than 2 times in the number of banded neutrophils, starting from admission of the victim (days 1–3 - $21.78 \pm 2.44\%$, days 5–7 - $22.43 \pm 1.94\%$, days 10–14 – $24.27 \pm 2.75\%$, $p < 0.05$). During the shock period, the victims developed moderate hypocoagulation (fibrinogen – 2.84 ± 0.54 g / l), which up to days 5–7 turned into moderate hypercoagulation (fibrinogen – 4.93 ± 0.41 g / l), which persisted. up to days 10–14 (fibrinogen – 6.27 ± 0.41 g / l), $p < 0.05$.

Comparative analysis of homeostasis indices in victims with combined extremely severe pelvic injury showed that all victims, both dead and survivors, on days 1–3 after admission, had severe respiratory and circulatory disorders (SVI – 24.14 ± 1.15 ml / m², CI – 1.84 ± 0.35 l / min / m², RI – 0.96 ± 0.10 RU, ITF – 74.15 ± 2.18 RU, $p < 0.05$, VTSI – 2.01 ± 0.06 RU, BSI – 42.18 ± 2.27 RU, $p < 0.05$), however on days 5–7 the majority of IBR indices in those, who survived, improved, although the prognosis remained "unfavorable" (SVI - 34.76 ± 1.18 ml / m², RI - 1.42 ± 0.08 units, ITF - 78.01 ± 0.35 units), VTSI – 1.39 ± 0.02 RU, BSI – 28.12 ± 0.28 RU, $p < 0.05$, CI – 1.84 ± 0.35 l / min / m², $p < 0.05$, and only starting from days 10–14, in the group of survivors there was a favorable prognosis, which was characterized by the approximation of IBR indices to the physiological norm (SVI - 51.06 ± 3.21 ml / m², CI - 3.41 ± 0.12 l / min / m², BSI - 27.03 ± 0.38 units, RI – 1.44 ± 0.07 RU, ITF – 79.14 ± 0.78 units, VTSI – 1.15 ± 0.01 RU, $p < 0.05$ in comparison with indices of the died victims group.

Thus, in UPIP victims with mild and severe trauma by the ATS scale with a favorable prognosis of traumatic disease, post-traumatic anemia was observed during the first 3-5 days after injury, and respiratory and circulatory disorders were fully restored up to days 7-10, which gives grounds to recommend performing invasive surgical interventions with indices on pelvic bones in this group of patients from days 5-7 of traumatic disease. Extremely severe trauma is characterized by severe anemia on days 5-7 after injury and normalization of cardiac activity and respiratory system up to days 10-15.

The question of the terms for internal osteosynthesis of unstable pelvic injuries in polytrauma, especially in type "C" fractures remains important. Ushakov S.A. et al., (2011) [7] in their studies showed the possibility of performing percutaneous transiliosacral screw fixation in posterior instability with minimal blood loss performed in the resuscitation phase of treatment. In the late 1990s, data were obtained on improving the treatment outcomes in patients who were subjected to "early" surgical treatment, especially for type "C" injuries [13]. However, other studies showed that patients who had undergone surgery at an early stage have a higher risk of secondary organ damage as a result of prolonged surgery (more than 6 hours), that significantly increases mortality and the incidence of multiple organ failure. According to studies performed by Rommens P.M. et al., (2002) [13], Pape H.C., Krettek C. (2003) [12], re-operation for pelvic injury should preferably be performed between days 5 and 10 after admission, rather than within 24 hours.

The most optimal is to perform invasive surgical interventions (internal metallic osteosynthesis - MOS, combined MOS of the unstable pelvic ring) in the early and late periods of TD up to day 21 after injury [9, 10]. According to our data, invasive surgical interventions in the pelvic bones in 2.94% of cases (MOS of the ventral pelvis during the pelvic organs operations) were performed on the 1st day after injury, in 51.47% - up to day 21 after injury, in 20. 59% - within the terms of over 21 days.

Conclusion

Thus, the severity of anatomical injuries and prediction of the UPIP victims' condition on admission should be more informatively assessed according to the proposed ATS scale (89.29% of statistical significance). In other periods of TD, in order to monitor the clinical course of injury and the choice of rational surgical treatment tactics, it is advisable to use our method for anatomical and functional assessment of the injury severity based on IBR by M.I. Tishchenko. Analysis of changes in homeostasis (indices of respiratory and circulatory disorders and laboratory indices of red blood) depending on the injury severity in patients with UPIP gives grounds to recommend the invasive surgery with indications for pelvic bones in this group of victims: for mild and severe injuries – from days 5-7 of traumatic disease, for extremely severe trauma from days 10–14 of TD with a favorable prognosis.

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Реферати

ВИБІР СТРОКІВ ХІРУРГІЧНОГО ЛІКУВАННЯ НЕСТАБІЛЬНОГО ТАЗОВОГО КІЛЬЦЯ У ПОСТРАЖДАЛИХ З ПОЛІТРАВМОЮ НА ОСНОВІ АНАТОМО-ФУНКЦІОНАЛЬНОЇ ОЦІНКИ ТЯЖКОСТІ ТРАВМИ

Бурлука В.В., Анкін Н.Л., Денисенко В.Н., Максименко М.А., Шепітько К.В.

Метою роботи було покращення результатів лікування постраждалих з нестабільними пошкодженнями таза при політравмі. Проведено дослідження показників інтегральної реографії тіла і деяких лабораторних показників крові у 137 постраждалих з нестабільними пошкодженнями таза при політравмі (НПТП). Усі постраждалі мали нестабільні пошкодження таза, а саме типи В і С за міжнародною класифікацією АО (M. Tile 1995). Ротаційно-нестабільні переломи (тип В) спостерігали у 101 (73,72%) випадків, вертикально-нестабільні (тип С) – у 36 (26,28%). Науково обґрунтовано, що тяжкість анатомічних пошкоджень і прогнозування стану постраждалих з НПТП при поступленні більш інформаційно оцінювати за шкалою ATS (89,29% статистичної достовірності). Проведення інвазивних операційних втручань, при показаннях, на кістах таза при нетяжкій і тяжкій травмі можливе з 5 – 7 доби травматичної хвороби, при вкрай тяжкій травмі – з 10 – 14 доби ТХ при сприятливому прогнозі, який визначався з урахуванням показників інтегральної реографії тіла.

Ключові слова: політравма, нестабільні пошкодження таза, інтегральна реографія.

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ВЫБОР СРОКОВ ХИРУРГИЧЕСКОГО ЛЕЧЕНИЯ НЕСТАБИЛЬНОГО ТАЗОВОГО КОЛЬЦА У ПОСТРАДАВШИХ С ПОЛИТРАВМОЙ НА ОСНОВЕ АНАТОМО-ФУНКЦИОНАЛЬНОЙ ОЦЕНКИ ТЯЖЕСТИ ТРАВМЫ

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Целью работы было улучшение результатов лечения пострадавших с нестабильными повреждениями таза при политравме. Проведено исследование показателей интегральной реографии тела и некоторых лабораторных показателей крови у 137 пострадавших с нестабильными повреждениями таза при политравме (НПТП). Пострадавшие имели нестабильные повреждения таза, а именно типы В и С по международной классификации АО (M. Tile 1995). Ротационно-нестабильные переломы (тип В) наблюдали у 101 (73,72%) случаев, вертикально-нестабильные (тип С) - у 36 (26,28%). Научно обосновано, что тяжесть анатомических повреждений и прогнозирование состояния пострадавших с НПТП при поступлении более информационно оценивать по шкале ATS (89,29% статистической достоверности). Проведения операционных вмешательств на костях таза при нетяжелой и тяжелой травме возможно с 5 - 7 суток травматической болезни, при крайне тяжелой травме - с 10 - 14 суток ТХ при благоприятном прогнозе, который определялся с учетом показателей интегральной реографии тела

Ключевые слова: политравма, нестабильные повреждения таза, интегральная реография.

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ASSESSMENT OF THE DIETARY ENERGY INTAKE OF YOUNG PEOPLE WITH NORMAL WEIGHT AND OVERWEIGHT

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The problem of weight gain and obesity with the formation of energy imbalance remains relevant especially among children and young people. The paper was aimed at determining the dietary energy intake of young people with normal weight and overweight and its compliance with energy needs. The study involved 84 male and female subjects aged 18-25. Height, body weight, waist circumference, and thighs circumference, and their ratio was determined. According to the body mass index (BMI), 22 women and 22 men (BMI 18.50-24.99 kg/m²) have been assigned to the control group and the group with overweight involved 20 men and 20 women (BMI 25.00-29.99 kg/m²). Nutritional status has been studied by the method of 24-hour nutrition reproduction by making records in the food diary during a weekday and weekend day. The value of the basal metabolic rate was calculated by the Mifflin-St Jeor equation. Was determined the recommended daily energy dietary intake maintains the existing body weight. The obtained data were processed statistically. The development of energy imbalance in people with overweight of both genders, a significant increase in the energy value of the dietary intake compared to controls and in comparison with the recommended daily energy intake has been established. The increase in energy value is accompanied by the altered structure of nutrients due to increased consumption of mainly fats by male subjects with overweight and fats and carbohydrates by female subjects compared to the controls. In men of the control group, the increase in energy value of the dietary intake relative to the recommended daily energy intake is less pronounced compared to subjects with overweight. Prolonged existence of the positive energy balance is the risk factor for the development of obesity and metabolic disorders.

Keywords: body mass index, overweight, energy value of dietary intake, energy metabolism, recommended daily dietary energy intake.

The work is a fragment of the research project "Comprehensive study of the pathogenetic role of M1 and M2 macrophages subpopulations in the development of chronic obstructive pulmonary disease for the development and justification of personalized therapy based on body weight", state registration No. 0117U005252.

Nutrition is essential for vital activity of the human body and is considered one of the most important factors determining its health, physical and psycho-emotional state. Over the last decade, the problem of high-calorie diet has increased significantly in Ukraine and worldwide, especially among children and young people [3, 5]. Reduce in physical activity due to technical progress and automation contributes to reduce in energy consumption, which in combination with excessive food consumption, disorder of the ratio of nutrients leads to energy imbalance, resulted in excessive energy substrates in the body, accumulated in the fat depot, which in turn leads to weight gain and obesity [14].

The formation of obesity is accompanied by the accumulation and activation of macrophages in the adipose tissue. Obesity activates pro-inflammatory M1 macrophages (classically activated), which stimulate leukocyte infiltration and reduces the fraction of M2 macrophages (alternatively activated) [1]. The phenotype of adipose tissue macrophages in obesity differs from the classical phenotype M1, since, along with the synthesis of proinflammatory cytokines, the profile of the superficial receptors is characteristic of the M2 phenotype. In normal-weight people, a subpopulation of M2 macrophages predominates, which secrete anti-inflammatory cytokines and use oxidative metabolism to maintain homeostasis of the adipose tissue [10].

Weight gain and obesity leads to the development of chronic systemic inflammation of low intensity, which contributes to the violation of the mechanisms of regulation of energy homeostasis [4]. The formation of a permanent energy imbalance is an unconditional trigger factor for the emergence of dangerous diseases in young age and adulthood. Notwithstanding numerous studies, the problem of energy imbalance with weight gain and obesity remains relevant to date; in recent years, an alarming increase in this pathology, especially among children and young people, has been noted.

The purpose of the paper was to determine the dietary energy intake of young people with normal weight and overweight and its compliance with energy needs.

Materials and methods. The study involved 84 male and female subjects aged 18-25. Informed consent was signed by each subject. The study was conducted with the permission of the Commission on Bioethics of the Ukrainian Medical Stomatological Academy. The anamnestic data of the subjects were recorded into the observation sheet.

Anthropometric measurements (height, body weight, waist circumference (WC) and thighs circumference (TC) and their ratio) were made. Body mass index (BMI) was calculated according to the equation: body weight (kg)/height (m²). According to the BMI, 22 women and 22 men (BMI 18.50-24.99

kg/m²) have been assigned to control group and the group with overweight involved 20 men and 20 women (BMI 25.00-29.99 kg/m²).

The assessment of the amount of consumed food was made using the 24-hour reproduction of nutrition with making records in the food diary during a weekday and weekend day [6]. The amount of consumed food with registration of the name of dishes and beverages, their composition, method of preparation, volume of liquid dishes was recorded. To determine the intervals between meals, the time and duration of intake, the last meal consumed before bedtime, was indicated. The volume of food was estimated using the album with photos of main dishes and beverages; the respondent chose the appropriate dishes. The nutrient content and energy value were determined using the tables.

The value of the basal metabolic rate (BMR) was calculated by the Mifflin-St Jeor equation [13]: for men: $BMR = 10.0 \times \text{body weight (kg)} + 6.25 \times \text{height (cm)} - 5.0 \times \text{age (years)} + 5$; for women: $BMR = 10.0 \times \text{body weight (kg)} + 6.25 \times \text{height (cm)} - 5.0 \times \text{age (years)} - 161$.

In addition, respondents answered the questions about the amount of physical activity per day, participation in sports clubs and sports activities on their own. The recommended daily energy dietary intake to maintain the existing body weight was calculated by the equation: Estimated Energy Requirement (EER) = BMR × PAC [12]; physical activity coefficient (PAC) of 1.2 corresponded to individuals leading the «sitting lifestyle».

The STATISTICA 10 software package (Stat Soft Inc, USA) was used for statistical processing of the resulting data. The arithmetic mean (M), its mean accuracy (m) was determined. Statistical significance was determined using the Student's t-test. The data was considered statistically significant at $p < 0.05$.

Results of the study and their discussion. Anthropometric values have been determined in subjects of both groups, additionally divided by gender (table 1). Increase in the values of the body weight, BMI, WC, TC and WC/TC ratio by 26.85%, 25.13%, 17.52%, 11.65% and 5.0%, respectively, have been noted in men with overweight compared to controls ($p < 0.05$). In women, the values of the body weight, BMI, WC and TC were by 23.53%, 25.66%, 16.67%, and 11.60%, respectively, higher compared to controls ($p < 0.05$) (табл. 1).

Table 1

Anthropometric values of the subjects of the study groups (M±m)

Parameters	Men		Women	
	with normal body weight n=22	with overweight n=20	with normal body weight n=22	with overweight n=20
Age, years	19.50 ± 0.34	19.93 ± 0.44	19.27 ± 0.32	19.25 ± 0.34
Height, cm	1.77 ± 0.01	1.78 ± 0.01	1.69 ± 0.01	1.67 ± 0.01
Body weight, kg	68.31 ± 1.71	86.65 ± 1.24*	62.09 ± 1.00	76.70 ± 1.60*
BMI, kg/m ²	21.65 ± 0.32	27.09 ± 0.28*	21.71 ± 0.19	27.28 ± 0.23*
WC, cm	75.90 ± 1.19	89.20 ± 0.83*	69.90 ± 0.70	81.55 ± 0.93*
TC, cm	94.18 ± 0.95	105.15 ± 0.92*	96.59 ± 1.00	107.80 ± 0.97*
WC/TC ratio	0.80 ± 0.01	0.84 ± 0.01*	0.72 ± 0.01	0.75 ± 0.01

Notes herein after in tables 2-3: * – $p < 0.05$ compared to individuals with normal body weight.

Anthropometric values in men and women with overweight were significantly higher compared to controls.

The method of 24-hour reproduction of nutrition has shown that during the weekday and weekend day the total mass of food consumed by men with overweight was significantly higher by 34.71% and 42.05%, respectively, compared to controls. On a working day and weekend day, the energy value of dietary intake of men with overweight was by 45.79% and 56.26%, respectively, higher compared to controls ($p < 0.05$) (table 2).

Table 2

Daily dietary intake of the subjects of study groups (M±m)

Parameters	Men		Women	
	with normal body weight, n=22	with overweight n=20	with normal body weight, n=22	with overweight n=20
Energy value, kcal, weekday	2261.62±69.82	3297.11±89.04*	1270.73±63.52	2080.34±115.28*
Proteins, g, weekday	84.17±6.97	119.53±9.95*	54.34±4.60	82.70±7.44*
Fats, g, weekday	91.67±5.13	152.07±9.06*	49.82±4.23	84.90±7.17*
Carbohydrates, g, weekday	277.64±13.34	366.26±17.70*	150.53±11.30	244.99±16.61*
Energy value, kcal, weekend day	2465.42±52.08	3852.36±139.69*	1739.51±97.89	2788.41±163.58*
Proteins, g, weekend day	88.26±4.67	124.30±10.59*	70.62±5.67	91.46±9.52*
Fats, g, weekend day	98.68±5.90	167.61±13.39*	70.28±5.46	132.82±11.56*
Carbohydrates, g, weekend day	308.84±17.47	451.00±21.28*	207.16±16.04	296.98±19.90*

Differences in nutrition pattern have been identified. It has been established that on a weekday, men with overweight consumed protein, fat and carbohydrates by 42.01%, 65.89% and 31.92%, respectively, higher compared to controls ($p<0.05$). On a weekend day, consumption of proteins, fats and carbohydrates was by 40.83%, 69.85% and 43.03%, respectively, higher compared to controls ($p<0.05$) (table 2).

On a weekday and weekend day, the total mass of food consumed by women with overweight was significantly by 25.77% and 17.10%, respectively, higher compared to controls. On a weekday and weekend day, the energy value of dietary intake of women with overweight was by 63.71% and 60.29%, respectively, higher compared to controls ($p<0.05$) (table 2).

On a weekday, women with overweight consumed protein, fat and carbohydrates by 52.19%, 70.41% and 62.75%, respectively, higher compared to controls. On a weekend day, consumption of proteins, fats and carbohydrates was by 29.51%, 30.14% and 43.35%, respectively, higher compared to controls ($p<0.05$) (table 2).

The findings of the study have shown that the rates of the energy value of daily dietary intake and consumption of essential nutrients of subjects with overweight of both genders were significantly higher compared to subjects with normal body weight.

At the next stage, the value of the basal metabolic rate and recommended daily dietary energy intake has been determined. The analysis of the data on the amount of daily physical activity has shown that almost all respondents with normal weight and overweight led a passive lifestyle and were not involved in physical activities. Therefore, the coefficient of 1.2 was used when calculating the recommended daily dietary energy intake.

The comparison of the values of the basal metabolic rate has shown that in male and female subjects with overweight it was by 10.71% and 9.46%, respectively, higher compared to controls ($p<0.05$). The similar differences have been noted in the rates of recommended daily dietary energy intake (table 3).

Table 3

The value of the basal metabolic rate and recommended daily dietary energy intake in the subjects of the study groups (M±m)

Parameters	Men	
	with normal body weight n=22	with overweight n=20
The basal metabolic rate, kcal/day	1655.63±49.31	1832.87±59.58*
Recommended daily dietary energy intake, kcal/day	1986.75±59.17	2199.45±71.50*
Parameters	Women	
	with normal body weight n=22	with overweight n=20
The basal metabolic rate, kcal/day	1421.67±17.14	1556.13±24.46*
Recommended daily dietary energy intake, kcal/day	1706.00±21.00	1867.35±29.35*

The comparison of the recommended daily dietary energy intake and its actual intake by the subjects with normal weight and overweight on a weekday and a weekend day has been made to determine the presence of the energy imbalance (table 4).

Table 4

Comparison of the energy value of daily dietary intake and recommended daily dietary energy intake in the subjects of the study groups (M±m)

Parameters	Men		Women	
	with normal body weight, n=22	with overweight n=20	with normal body weight, n=22	with overweight n=20
Recommended daily dietary energy intake, kcal/day	1986.75±59.17	2199.45±71.50	1706.00±21.00	1867.35±29.35
Energy value, kcal weekday	2261.62±69.82*	3297.11±89.04*	1270.73±63.519	2080.34±115.28*
Energy value, kcal weekend day	2465.42±52.08*	3852.36±139.69*	1739.51±97.89	2788.41±163.58*

Notes: * - $p<0.05$ – compared between the energy value and recommended daily dietary energy intake

On weekend day and weekday, the energy value of dietary intake in men with normal body weight was by 13.84% and 24.09% ($p<0.05$), respectively, higher than recommended daily dietary energy intake (coefficient of physical activity = 1.2) (table 4).

On weekend day and weekday, the energy value of dietary intake in men with overweight was by 49.91% and 75.15% ($p<0.05$), respectively, higher than recommended daily dietary energy intake (table 4).

On weekend day and weekday, the energy value of dietary intake in women with overweight was by 11.41% and 49.32% ($p < 0.05$), respectively, higher than recommended daily dietary energy intake (coefficient of physical activity = 1.2) (table 4).

Thus, the findings of the study have shown a significant increase in the energy value of dietary intake in the overweight subjects of both genders compared to controls and in comparison with the recommended daily energy intake. The established increase in the energy value of the dietary intake relative to the recommended daily energy intake in men of the control group was less pronounced than in overweight subjects.

Current data show that even insignificant systematic increase in energy balance by 1-2% of daily energy consumption can lead to considerable long-term changes in body weight. It is believed that only 10-20 kcal per day of excessive energy consumption is enough to gain 0.5-1 kg per year by an adult with a deficit of its use. The programs of National Health and Nutrition Examination Survey (NHANES) report about significant increase in energy consumption along with the rapid increase of obesity in people of different age-groups [11].

The assessment of the nutritional status of the adult population of Ukraine in current socio-economic conditions showed an excess of energy value of dietary intakes in 38.4% of people. The increase in body mass index of rural population by 34.5% and urban population by 42.4% is noteworthy [7].

Our studies have shown that the energy value of the actual dietary intake in overweight subjects of both genders is significantly higher compared to controls and the recommended daily energy intake.

Notably, in male subjects with normal body weight, the energy value of the dietary intake on a weekday and weekend day significantly exceeded the recommended daily energy intake. Despite the fact that their positive energy balance was much lower than in subjects with overweight (13.84% vs. 49.90% on a weekday and 24.09% vs. 75.15% on a weekend day, respectively), the resulting data are worth considering. In our opinion, first of all young people should reconsider their lifestyle. Given that the level of physical activity of the subjects is very low, the long-term existence of a positive energy balance can lead to a gradual weight gain.

The comparison of the structure of nutrients in the daily dietary intake revealed differences between the groups with normal weight and overweight. Thus, in male subjects with overweight there was an increase in consumption of mostly fats on a weekday and weekend day and in females, mostly fats and carbohydrates, compared to controls.

The studies show that a diet high in carbohydrates or an increase in the percentage of total energy intake in the form of carbohydrates significantly increases the risk of obesity. The relationship between obesity and long-term consumption of food high in unrefined carbohydrates and fat has been established [15].

The study revealed non-rationed nutrition of subjects with overweight, unusual timing of food eating, failure to keep to intervals between the last meal and sleep.

Chernova N.N., et al (2017) report that up to 80% of students consume most of their food after 18:00 or just before bedtime, when the main caloric and substantial meal is consumed in the evening. This dietary pattern can lead to weight gain and contribute to the development of diseases of the gastrointestinal tract and endocrine system. Moreover, students' dietary intake consist of mainly products rich in carbohydrates and fats of animal origin with inadequate ratio of proteins, fats and carbohydrates [8].

It has been previously found that energy imbalance in young people is the key factor for the development of excess energy with accumulation in the fat depot and weight gain due to significant prevalence of the energy value of the dietary intake over energy expenditure, as well as other factors as eating disorders, changes in quality of life, increased basal metabolism, insulin resistance, changes in markers of chronic systemic inflammation and neuropeptides [9].

Our investigations also showed the negative impact of weight gain on the quality of life of young people with overweight, in particular, a decrease in physical functioning, role physical and emotional functioning, pain, general health and mental health in male subjects, as well as lower rates physical functioning, general health, pain intensity, viability, social and role emotional functioning, mental health and mental health component in female subjects [2].

Generally, the relationship between the accumulation of excess energy in the form of adipose tissue and low-intensity chronic inflammation can lead to the development of chronic diseases, accompanied by metabolic disorders, cardiovascular and endocrine disorders. Lifestyle changes, sufficient physical activity, correction of behavioral factors can reduce the level of chronic systemic inflammation, which is of great clinical importance [4].

Conclusion

The development of energy imbalance in people with overweight of both genders, a significant increase in the energy value of the dietary intake compared to controls and in comparison with the recommended daily energy intake has been established. The increase in energy value is accompanied by the altered structure of nutrients due to increased consumption of mainly fats by male subjects with overweight and fats and carbohydrates by female subjects compared to the controls. In men of the control group, the increase in energy value of the dietary intake relative to the recommended daily energy intake is less pronounced compared to subjects with overweight. Prolonged existence of the positive energy balance is the risk factor for the development of obesity and metabolic disorders.

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Реферати

**ОЦІНКА ЕНЕРГЕТИЧНОЇ ЦІННОСТІ
ХАРЧОВОГО РАЦІОНУ В ОСІБ МОЛОДОГО
ВІКУ З НОРМАЛЬНОЮ І ПІДВИЩЕНОЮ
МАСОЮ ТІЛА**

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Проблема підвищення маси тіла і ожиріння з формуванням енергетичного дисбалансу залишається актуальною особливо серед дітей і людей молодого віку. Метою нашого дослідження стало визначення енергетичної цінності харчового раціону у осіб молодого віку з нормальною і підвищеною масою тіла та її відповідності енергетичним потребам. Обстежено 84 особи обох статей віком від 18 до 25 років. Визначена маса тіла, зріст, обхват талії, стегон, їх співвідношення. За індексом маси тіла (ІМТ) сформовані групи: контрольна по 22 особи (ІМТ 18.50-24.99 кг/м²) і група з підвищеною масою по 20 осіб обох статей (ІМТ 25.00-29.99 кг/м²). Харчовий статус досліджували методом 24-годинного відтворення харчування із заповненням харчового щоденника за робочий і вихідний дні. Розраховували величину основного обміну енергії за формулою Міффіліна-Сан-Джеора та рекомендоване добове споживання енергії для підтримки існуючої маси тіла. Отримані дані оброблені статистично. Визначено достовірне підвищення енергетичної цінності харчового раціону у осіб обох статей з підвищеною масою тіла у

**ОЦЕНКА ЭНЕРГЕТИЧЕСКОЙ ЦЕННОСТИ
ПИЩЕВОГО РАЦИОНА У ЛИЦ МОЛОДОГО
ВОЗРАСТА С НОРМАЛЬНОЙ И ПОВЫШЕННОЙ
МАССОЙ ТЕЛА**

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Проблема повышения массы тела и ожирения с формированием энергетического дисбаланса остается актуальной особенно среди детей и людей молодого возраста. Целью нашего исследования стало определение энергетической ценности пищевого рациона у лиц молодого возраста с нормальной и повышенной массой тела и ее соответствия энергетическим потребностям. Обследовано 84 человека обоих полов в возрасте от 18 до 25 лет. Определена масса тела, рост, обхват талии, бедер, их соотношение. По индексу массы тела (ИМТ) сформированы группы: контрольная по 22 человека (ИМТ 18.50-24.99 кг/м²) и группа с повышенной массой по 20 человек обоих полов (ИМТ 25.00-29.99 кг/м²). Пищевой статус исследовали методом 24-часового воспроизведения питания с заполнением пищевого дневника за рабочий и выходной дни. Рассчитывали величину основного обмена энергии по формуле Миффлина-Сан-Джеора и рекомендованное суточное потребление энергии для поддержания существующей массы тела. Полученные данные обработаны статистически. Определено достоверное повышение энергетической ценности пищевого рациона у лиц обоего пола с повышенной массой тела по сравнению с

порівнянні з контрольною групою та при порівнянні з рекомендованим добовим споживанням енергії. Підвищення енергетичної цінності супроводжується зміною структури нутрієнтів за рахунок підвищеного споживання переважно жирів особами чоловічої статі з підвищеною масою тіла та жирів і вуглеводів особами жіночої статі у порівнянні з контрольною групою. У чоловіків контрольної групи підвищення енергетичної цінності харчового раціону відносно рекомендованого добового споживання енергії менш виражене, ніж у осіб з підвищеною масою тіла. Тривале існування позитивного енергетичного балансу є фактором ризику розвитку ожиріння і метаболічних порушень.

Ключові слова: індекс маси тіла, підвищена маса тіла, енергетична цінність харчового раціону, енергетичний обмін, рекомендоване добове споживання енергії.

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контрольної групою и при сравнении с рекомендованным суточным потреблением энергии. Повышение энергетической ценности сопровождается изменением структуры нутриентов за счет повышенного потребления преимущественно жиров лицами мужского пола с повышенной массой тела и жиров и углеводов лицами женского пола по сравнению с контрольной группой. У мужчин контрольной группы повышение энергетической ценности пищевого рациона относительно рекомендованного суточного потребления энергии менее выраженное, чем у лиц с повышенной массой тела. Длительное существование положительного энергетического баланса является фактором риска развития ожирения и метаболических нарушений.

Ключевые слова: индекс массы тела, повышенная масса тела, энергетическая ценность пищевого рациона, энергетический обмен, рекомендованное суточное потребление энергии.

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MORBIDITY AND PREVALENCE OF THE NERVOUS SYSTEM DISEASES IN CHILDREN OF UKRAINE

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The article presents clinical and epidemiological data on the prevalence and incidence of the nervous system diseases (hereinafter – NSD) in all regions of Ukraine from 1993 to 2019. The incidence rates of NSD in children from regions with radiological control areas were higher than the nation-wide ones and the incidence rates in children from other regions by 15.9%. The incidence of NSD in children affected by the Chernobyl accident was higher than the nation-wide by 44.5%, which does not permit to exclude the direct and indirect effects of radiation. The results of our observations on the incidence of the NSD in children of Ukraine indicate that children suffered prolonged exposure to ecotoxic factors, including radiation, have higher levels of prevalence and morbidity of the NSD, which requires specialists' attention and effective medical and diagnostic measures.

Key words: children, disability, nervous system diseases, Chernobyl disaster.

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The problem of morbidity and prevalence of the NSD in children remains relevant worldwide due to the annual increase in disability due to this pathology and the social significance of this issue [4, 7, 10]. Significant advances in the diagnostics and treatment of the NSD, including those in the spheres of neurogenetics, neuropharmacology, neuroimmunology, molecular biology, studies of nervous system's metabolism, have significantly changed the views of scientists and practitioners about the effects of environmental factors on the development and functioning of nervous system. It has also led to a better understanding of the neurobiological basis for developmental delay, cerebral palsy (hereinafter – CP), autism and demyelinating diseases [11, 13, 14].

Thus, Yevtushenko SK. (2016) note that the detection of cerebrovascular pathology is spreading every year, including in children, which is associated with abnormalities and diseases of the cardiovascular system, diabetes, vasculitis and complications due to infectious diseases [3]. This thesis sounds more relevant than ever due to the rapid spread of COVID-19 in the world.

In Ukraine, over the past 25 years, the situation with the general morbidity of children, including NSD, has changed significantly due to the influence of a number of social and environmental factors [2, 8]. One reason of this kind was the accident at the Chernobyl nuclear power plant in 1986, which led to direct and indirect radiation exposure of more than 4 million people [8]. The extent of its environmental, medical and social impact on the health of the population and the life quality of victims and those living in contaminated areas is difficult to determine today [1, 6].

The WHO emphasizes that the quality of the environment is a direct and indirect factor that determines the level of human health, and the deterioration of the environment leads to a decrease in health and quality of life. It can also lead to more than 80 diseases and types of injuries [10, 12]. Research works

on the assessment of radiation exposure effect on the nervous system status (hereinafter – CNS) mainly concerns the assessment of the growth of the nervous system's congenital malformations in the offspring of irradiated parents. The results of the study on the CNS in liquidators indicates a variety of clinical manifestations involving various CNS structures in the pathology [9]. Irradiated children were more likely to have NSD and mental disorders. They had a lower overall IQ due to a lower verbal IQ and increased of disharmonious mentality [8].

The purpose of our study was to assess changes in the morbidity and prevalence of NSD among children of Ukraine over the past 25 years to determine the possible impact of adverse environmental factors, including the impact of the Chernobyl accident (hereinafter – ChAcc) on their development.

Materials and methods. Analysis was performed on the dynamics of the morbidity and prevalence of the NSD in children from different regions of Ukraine, especially in those with areas of radiological control (hereinafter – ARC), formed more than 30 years ago as a result of the ChAcc. Methods of statistical assessment and epidemiological analysis of data presented by the Center for Medical Statistics of the Ministry of Health of Ukraine from 1993 to 2019 were used [5].

Statistical estimation methods were used, in particular U-criterion of sign ranks (Wilcoxon-Mann-Whitney test) to compare the morbidity indices of NSD children from the same regions of Ukraine at different time intervals and in different study groups. A statistical analysis was carried out on the morbidity and prevalence of NSD in the following groups of children: the total number of children in the country; children living in regions with areas of radiological control; children living in other regions of the country; children who are classified as victims of the ChAcc.

Cluster assessment of the regions of Ukraine according to the morbidity levels of children with NSD was carried out by the method of k-means due to the ratio of morbidity levels among children with NSD to the nation-wide index and the distribution of regions in the country according the ARC presence. The correlation between the amount of lead emissions into the atmosphere and the number of newly diagnosed cases of NSD in children was estimated. Lead linear relationship and to determine the correlation and possible relationship between lead emissions from stationary air pollution sources and the incidence emissions were determined according to the data of the annual statistical collection of the State Statistics Service of Ukraine for 2017 [12].

The Spearman's rank correlation coefficient was used to establish and prevalence of NSD in children from different regions. Statistical processing of the study results was performed using the software product STATISTICA 6.1 and Excel-2010.

The study was carried out in compliance with the main provisions of the ICH GCP and the Helsinki Declaration on the Ethical Principles of Medical Research Relating to Human Subjects and its Revisions (Seoul, 2008), the Council of Europe Convention on Human Rights and Biomedicine (2007), recommendations of the Bioethics Committee at the Presidium of the National Academy of Medical Sciences of Ukraine (2002) and the relevant meeting of the Ethics Committee at the Bogomolets National Medical University.

Results of the study and their discussion. According to the Center for Medical Statistics of the Ministry of Health of Ukraine, the morbidity in children aged 0-17 years for the NSD in 2017 amounted to 132,354 new cases of NSD or 17.38 per 1000 children (of which NSD were first detected in 21 807 children of the first year of life or in 60.93 cases per 1000 people).

In 1993, 458944 cases of NSD were first detected, or 42.1 per 1,000 children aged 0-14 years. Note that over 25 years, the rate of reduction in the NSD morbidity in children aged 0-14 years was 73.9%. It is of interest that the adult population had a different trend – there was an increase in neurological pathology in the country almost twice [5].

The positive trend in the lower morbidity and likelihood of chronic neurological pathology in children of early preschool and school age may be due to the positive impact on their health through the use of modern perinatal technologies. However, the decrease in the incidence of could be due to a decrease in the level of diagnosing NSD, as well as due to reduced availability of specialized healthcare facilities in the regions, including remote and mountainous areas. This indicates the need to staff the pediatric neurological service of Ukraine and to improve the provision of specialized health care facilities with the necessary medical equipment.

The prevalence of NSD in children was 390,999 cases or 51.34 per 1,000 population. In 1993, the total of 790,338 cases of NSD were detected, or 72.5 per 1,000 children. Note that over 25 years, the rate of decrease in the prevalence of NSD in children aged 0-14 years was – 29.2%. 97,863 cases of NSD or 30.97 per 1,000 population were registered in children aged 0 to 6 years.

The maximum detection of children NSD was observed in children aged 7-14 years, where it amounted to 164,815 cases or 48.83 per 1,000 population. This amounted to 42.15% of the total number of registered NSD in all age groups. In adolescence, there was a maximum prevalence of NSD in children. The total of 128,321 cases or 118.8 per 1,000 population were registered, representing 32.8% of the total

number of registered NSD in all age groups. Within the same age group, the incidence of NSD was 33094 of newly diagnosed NSD cases or 30.06 per 1000 children, which was twice exceeding the level of young and school-age children.

Data on the increase in the prevalence of diseases with age due to the accumulation of chronic diseases are confirmed by the age-related change in the index of NSD accumulation – 2.02 among children including those aged 0–6 years, 3.24 among children aged 7–14 years and 3.87 among adolescents.

The structure of neurological diseases in childhood is now dominated by perinatally caused injuries of the nervous system, CP, epilepsy and epileptic syndromes, infectious impairments of the nervous system, the effects of postnatal traumatic brain injury, hereditary diseases of the nervous system, metabolic diseases with the nervous system impairment [3, 4].

Among children in 2017, 634 cases of CP or 0.08 per 1,000 population were first detected, of which 36 cases were in children under one year. The prevalence of this complex pediatric neurological pathology among infants was 16,099 cases or 2.11 per 1,000 population. Among the etiology of this pathology in half of the cases there are factors associated with perinatal pathology – miscarriage, abnormal pregnancy, complicated childbirth, neonatal diseases. Currently, the world mean incidence of CP is 2.5 per 1 thousand children [3]. This testifies to the efficacy of early prenatal diagnosis of such conditions in Ukraine and the proper potential of the pediatric neurological service.

Also, 3,014 cases of epilepsy or 0.40 per 1,000 population were detected in children. The prevalence of this pathology was 23,583 people or 3.1 per 1,000 population. Last year, 11,644 children in hospitals were diagnosed with a traumatic brain injury (680 of them were cases in children under 1 year of age); 1788 children were diagnosed with infectious-allergic impairment of the CNS (129 of them in children under 1 year); in 1469 – hypertension; 639 was diagnosed with cerebrovascular disease. 74 children, 20 of them under 1 year of age, were treated with a diagnosis of acute cerebrovascular accident of hemorrhagic type in the country's hospitals. Of these, 13 children died, 4 of them – under the age of 1. Ischemic type Acute Cerebrovascular Event was observed in 8 children, two of whom died. 5 children underwent treatment for cerebrovascular disorders. 146 children who were hospitalized in 2019 were diagnosed with multiple sclerosis.

In total, 102 children died in hospitals in 2019 as a result of NSD, including 36 children under 1 year of age. In the list of rankings for the incidence of childhood diseases, NSD ranked the 8th out of 17 diseases with a share of 2.94%. In 75% of cases, the onset of neurological pathology coincides with the perinatal period [2, 7]. Almost 60% of children with disabilities from childhood are children with the NSD: epilepsy, muscular dystrophies, CP, congenital central nervous system abnormalities, etc. [4, 7].

Thus, in 2019, 25,422 children with disabilities due to NSD were first registered in Ukraine, or 33.5 per 1,000 children. Disability was first established for 181 children with NSD, which is 0.24. Among other causes of disability, NSD with a share of 15.5% are third after congenital anomalies (share – 30%) and mental and behavioral disorders (share – 16.5%). The structure of disability due to NSD was dominated by CP – 12,608, epilepsy – 3,470, muscle atrophy – 1,472 and multiple sclerosis – 248 cases.

It should be noted that the highest rates of children's disability due to NSD were observed in Chernihiv region – 4.9 per 1,000 children; Transcarpathian – 4.77; Mykolaiv – 4.56; Cherkasy – 4.19. Among the 9 regions with areas of radiological control (hereinafter – ARC) in 7 regions, the level of disability due to NSD exceeded the national mean.

In 1993, the disability rate due to NSD was 38.2 per 1,000 children, which is almost slightly higher than today. The share of disability due to NSD has decreased by more than 2.5 times for 25 years, which is obviously the result of the effective implementation of modern perinatal technologies and early intervention strategies in the country [6, 8].

The largest number of NSD in 2017 was first detected in Kyiv region (26.97), Dnipropetrovsk region (24.03), Zhytomyr region (23.74), Kharkiv region (23.77), Rivne region (22.86), Ivano-Frankivsk region (22.10), Sumy (20.45). Of these regions, five – Kyiv, Zhytomyr, Rivne, Sumy and Ivano-Frankivsk – are the regions that have areas for radiological control due to contamination caused by the Chornobyl disaster. Other regions (Dnipropetrovsk and Kharkiv) with a high incidence of NSD have a developed industrial and agricultural sector with a significant load of pollutants on the environment and the child's body from 205 to 16 kg per a person, respectively [12].

The lowest levels of NSD incidence were observed in Khmelnytsky and Cherkasy regions (12.63 per 1,000 population), Poltava (12.64), Luhansk (13.06), and Ternopil (13.49) regions. In total, in 5 out of 9 regions with ARC, the incidence of NSD exceeded the national mean.

In 1993, in 7 out of 9 regions with ARC, the incidence of NSD was higher than the national mean. That is, 25 years ago, among the leading regions in terms of morbidity levels were numerically more regions with areas of radiological control. In general, the incidence of the NSD in children living in regions with areas of radiological control in the early 90's exceeded the national rates and incidence in children with NSD living in other regions of the country that did not have ARC (fig. 1).

In 339,278 children affected by the ChAcc, 8,998 cases of NSD were first detected, or 23.2 per 1,000 children, which is almost by 1.3 times more than the incidence of NSD children in Ukraine. The prevalence of NSD in children affected by the ChAcc was also high – it was 86.16 per 1,000 population and also significantly exceeded the national rate – 51.34. Among the children affected by the ChAcc, there was a high incidence of vegetative-vascular dysfunction – 4733 or 12.2 per 1000 children. The prevalence of these conditions was 206,501 cases or 53.12 per 1,000 children. Clustering of Ukrainian regions depending on the incidence of NSD and distribution of regions is given in table 1.

Low incidence of NSD in children from Cherkasy, Chernihiv, Poltava, Ternopil and Luhansk regions can be explained by insufficient detection of neurological pathology due to shortage of specialists and problems with implementation of modern medical and diagnostic technologies into the medical practice. At the same time, the leading regions in terms of the NSD prevalence are Dnipropetrovsk, Kharkiv region and the city of Kyiv, where the detection of neurological pathology is much better due to the availability of staff and equipped pediatric clinics and maternity hospitals. In addition, the possible increase in neurological pathology in children is associated with significant industrial potential and high emissions into the atmosphere, which has a negative impact on the environment [15].

Table 1

Regions of Ukraine clustering by morbidity levels of the NSD

Morbidity level in children with NSD	Regions with areas of radiological control	Regions without areas of radiological control
Areas with children's NSD incidence exceeding the national mean	Kyiv, Zhytomyr, Rivne, Ivano-Frankivsk	Dnipropetrovsk, Kharkiv
Areas with children's NSD incidence close to the national mean	Sumy, Vinnytsia, Volyn	City of Kyiv, Transcarpathian, Mykolaiv, Kherson, Odesa, Khmelnytsk, Donetsk, Lviv, Chernivtsi
Areas with children's NSD incidence lower than the national mean	Cherkasy, Chernihiv	Zaporizhzhia, Luhansk, Poltava, Ternopil

As shown in fig. 1, the incidence of NSD in children aged 0-14 years in Ukraine has decreased reliably by 73.9% ($p < 0.05$). The time decrease in the prevalence index of NSD by 29.2% was unreliable ($p > 0.05$). The same trend was characteristic of NSD incidence in children from regions with areas of radiological control, which decreased by 62.70% and reached its peak value of 45.3 per 1,000 children in 1993. A decrease in the incidence of NSD by 66.7% was observed in children affected by the ChAcc. Its peak value of 70.0 per 1,000 children was observed in 1993.

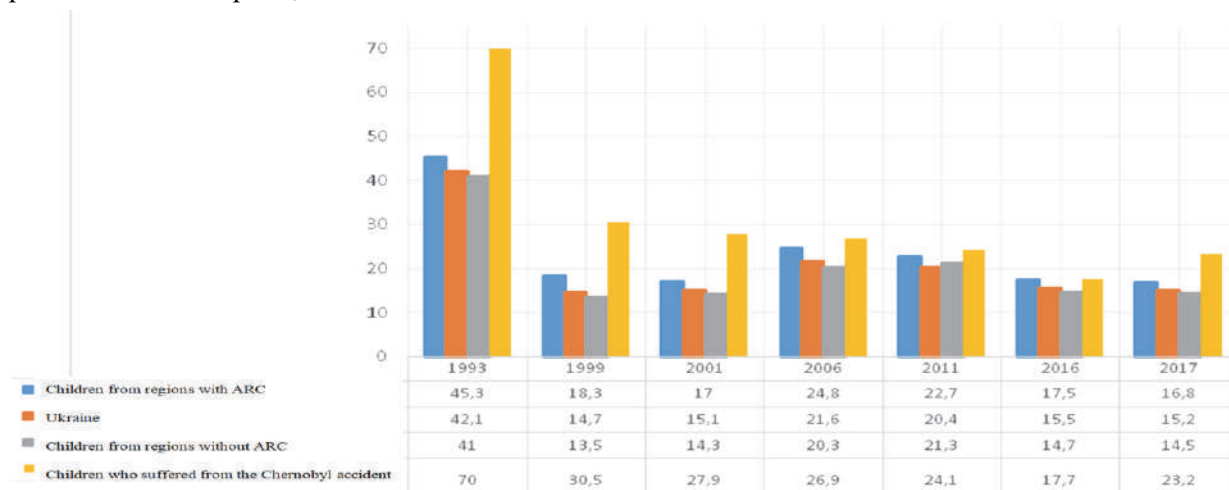


Fig. 1. Comparative dynamics of the incidence rates of the NSD in children of Ukraine, children affected by the ChAcc, children from regions with ARC, regions without ARC (1993 – 2017).

It should be noted that for more than 20 years of observation in Cherkasy, Zaporizhzhia, Chernivtsi regions there was the largest decrease in the incidence of NSD in children, respectively, – 87.3%, – 81.8%, – 80.7% compared to the incidence of NSD in 1993. At that time, in Kyiv and Zhytomyr regions the decrease in morbidity was within the range of – 21.5% – 29.8%. It is no coincidence that in these two regions with radiological control areas, a high incidence of NSD in children was observed during all the 25 years.

The incidence of NSD in children from the regions with ARC was higher than the national indices by 10.5% ($p > 0.05$) and exceeded the incidence in children from other regions by 15.9% ($p > 0.05$). The incidence of NSD in children affected by the ChAcc was higher by 47.3% than the national incidence of this pathology and by 38.1% of the incidence in the areas with ARC (fig. 1).

Apparently, children affected by the ChAcc had a high incidence of NSD during all the 25 years of observation, which significantly exceeded the rates of children from all observation groups ($p < 0.05$).

This indicates that the impact of the ChAcc on their bodies persisted in the second generation of children from parents who suffered as a result of the ChAcc. Comparing the incidence of NSD between radiation-contaminated areas and other areas, it is necessary to conclude that the main factors in the development of NSD at the moment are a set of social, psychological, environmental, hereditary and other factors. At the same time, even in contaminated areas, the role of the radiation factor decreases over time. However, the overall incidence rate of children from regions with ARC significantly exceeds the national rate [1].

A completely different and clearer picture of the ratio of NSD incidence rates in these contingents of children was observed in 1993, when the rates of children affected by the ChAcc were 66.2% higher than the national indices and by 54.5% higher than the incidence of NSD in children from radiation-contaminated areas, which in their turn exceeded the incidence of children from “conditionally” clean areas by 15.8%.

We have assessed the possible correlations between the incidence of NSD and the emissions volume for one of the most toxic to the nervous system pollutants – lead into the atmosphere from stationary and mobile sources of pollution. Thus, in 2019, according to the State Statistical Service of Ukraine, almost 70 tons of lead were discharged into the atmosphere, mostly from vehicles (in 2005 there was a maximum of lead emissions of 251.4 tons) [12]. According to the analysis, it was found that the Spearman correlation coefficient (ρ) is equal to 0.559, and the relationship between the studied features was direct and with a noticeable strength of the connection by the Chaddock scale. The number of degrees of freedom (f) was 10, but the critical value of Spearman's criterion for this number of freedom degrees is 0.587, which indicated a statistically insignificant relationship ($p > 0.05$) between the incidence of NSD and emissions of lead into the atmosphere, which in these time period also decreased due to the economic slowdown and the war in the East.

According to our study results, we report a decrease in the growth rate of NSD in children living in the areas with ARC and in children affected by the ChAcc. Obviously, this is the result of the advanced medical and diagnostic technologies implemented into modern medical practice, the effective treatment of the NSD in newborns. It is also possible that this is a consequence of the global reduction of pollutant emissions into the air of Ukraine, in particular neurotoxic lead and the reduction in time of the polluted environment's ecotoxic effects, particularly long half-life radionuclides after the ChAcc.

Conclusions

Over the last 25 years, there has been a 73.9% decrease in the incidence of NSD in children of Ukraine (0–14 years). Also in recent years, the prevalence of this pathology has decreased by 29.2%. Among children with the status of victims of the ChAcc, the incidence of NSD also decreased, but all years of monitoring exceeded the national indices of children with NSD and those living permanently in areas of the country that were contaminated by the ChAcc in 1986 year.

In children living permanently in the regions of the country that were contaminated by the ChAcc in 1986, the incidence of NSD exceeded the national rates in the early 90s and throughout all 25 years of monitoring, which requires the necessary medical and diagnostic measures in these regions.

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Реферати

ЗАХВОРЮВАНІСТЬ ТА ПОШИРЕНІСТЬ ХВОРОБ НЕРВОВОЇ СИСТЕМИ У ДІТЕЙ УКРАЇНИ

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У статті наведені клініко-епідеміологічні дані поширеності та захворюваності дітей на хвороби нервової системи в усіх областях України. Показники захворюваності на неврологічну патологію у дітей із території радіологічного контролю були більшими, ніж загальнодержавні та показники захворюваності у дітей з інших областей, що не дає можливості виключити прямий та опосередкований вплив радіаційного фактору. Результати наших спостережень свідчать, що у дітей, які зазнають тривалого впливу екотоксичних факторів, зокрема і радіаційного, спостерігаються більш високі рівні поширеності та захворюваності на неврологічну патологію, що потребує моніторингу фахівців та розробки ефективних лікувально-діагностичних заходів.

Ключові слова: діти, інвалідність, хвороби нервової системи, Чорнобильська катастрофа.

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ЗАБОЛЕВАЕМОСТЬ И РАСПРОСТРАНЕННОСТЬ БОЛЕЗНЕЙ НЕРВНОЙ СИСТЕМЫ У ДЕТЕЙ УКРАИНЫ

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В статье приведены клиничко-эпидемиологические данные распространенности и заболеваемости детей патологией нервной системы во всех областях Украины. Показатели заболеваемости неврологической патологией у детей из территории радиологического контроля были выше общегосударственных и показателей заболеваемости у детей из других областей, что не позволяет исключить прямого и опосредованного влияния радиационного фактора. Результаты наших наблюдений свидетельствуют, что среди детей, имеющих длительное воздействие экотоксических факторов, в том числе радиационное, наблюдаются более высокие уровни распространенности и заболеваемости неврологической патологией, что требует мониторинга специалистов и разработки эффективных лечебно-диагностических мероприятий.

Ключевые слова: дети, инвалидность, болезни нервной системы, Чернобыльская катастрофа.

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POSTMENOPAUSAL PERIOD - AS A RISK FACTOR FOR THE DEVELOPMENT OF THE REPRODUCTIVE SYSTEM TUMORS

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The study included a retrospective analysis of the medical histories of 301 women with various tumors of the reproductive system (Average age 61.6±0.4 years). The prospective study included 306 women with benign and malignant genital tumors in the postmenopausal period (Average age 59.3±0.4 years). All patients underwent genital echography; the levels of steroid hormones and blood biochemical parameters were studied; indicators of the cancer marker CA-125 were determined. It was found that in patients with ovarian cancer, the body mass index was 28.7±0.6 kg / m², in endometrial cancer 32.7±1.0 kg/m², in benign ovarian neoplasms, the BMI was in the range of 27.3±2.0 kg/m², in patients with endometrial hyperplastic processes 31.9±0.8 kg / m². Patients with ovarian cancer and endometrial cancer had a high rate of artificial termination of pregnancy (29.2% and 46.8%, respectively). Thus, risk factors for the development of neoplastic processes of genitalia in postmenopausal period are: the increase in body mass index, presence of infertility in anamnesis, the presence of gynecological and endocrine diseases in the reproductive and perimenopausal periods, high frequency of induced abortion in anamnesis.

Key words: postmenopausal period, ovarian cancer, endometrial cancer, endometrial hyperplastic processes, body mass index.

The work is a fragment of the doctoral dissertation: "Pathogenetic mechanisms, clinic and modern methods of diagnostics of reproductive organ tumors in the postmenopausal period".

According to the research results, by 2030, 1 billion 200 million women will be in the postmenopausal period, and by 2060, the number of postmenopausal women will be approximately 59.8% of the total female population in the world [11]. The process of the ovaries functional activity extinction is accompanied by physiological hypoestrogeny and, accordingly, depletion of the ovarian follicular apparatus and apoptosis of germ cells (with the presence of spontaneous genetic breakdowns) [7, 8]. This process is physiological in nature and is a natural process of the body aging.

In 2011 the working group of STRAW experts determined the parameters of the female body's life periods [2]. It was found that 20% of women have peri - and postmenopausal periods have a physiological

course. 50% of women have hypoestrogenia, accompanied by climacteric disorders of varying severity and characterized by polymorphism of clinical manifestations [14, 9].

It is established that in the postmenopausal period in the structure of gynecological morbidity, pathological processes of the endometrium, including endometrial cancer, occupy one of the leading places. At the same time, in the postmenopausal period, the probability of endometrial cancer developing reaches 30% [5, 15].

According to recent studies, it has been determined that relative and absolute hyperestrogeny plays a significant role in the development of endometrial hyperplastic processes in the postmenopausal period. Hormonal rearrangement of women's body against the background of ovarian hypofunction is a predisposing factor for the development of endometrial cancer. It should be noted that in postmenopause, the presence of diffuse and focal changes in the endometrium is characterized by an asymptomatic course [6, 10].

The authors' study revealed that in the pathogenesis of endometrial neoplastic processes, with prolonged postmenopause, the value of hyperestrogenia as such decreases, and the role of other factors is under study.

In recent years, there has been a significant increase in the incidence of ovarian cancer. Peri- and postmenopausal periods are risk factors for ovarian cancer [1, 11].

It should be noted that there is limited and contradictory information about the pathogenetic mechanisms and risk factors for the development of tumors of the reproductive system in the postmenopausal period. There is no data on the severity of clinical manifestations, criteria for modern methods of diagnosis of neoplasia, as well as the structure of tumors of the reproductive system in the postmenopausal period in the Republic of Azerbaijan.

The purpose of the study was to determine the risk factors for developing genital tumors in the postmenopausal period.

Materials and methods. The study included a retrospective analysis of the medical histories of 301 women with various tumors of the reproductive system. The prospective study included 306 women with benign and malignant genital tumors in the postmenopausal period. All patients were treated at the Oncology Clinic of the Azerbaijan Medical University and the National Cancer Center of the Republic of Azerbaijan. The average age of the examined women included in the prospective study was 59.3 ± 0.4 years. According to a retrospective study, the age of women was in the range of 61.6 ± 0.4 years.

The duration of the postmenopausal period in patients included in the prospective study was in the range of 9.8 ± 0.4 years.

This study included conducting a clinical examination of patients, determining the severity of menopausal syndrome on the Kupperman scale. All patients underwent transvaginal ultrasound examination of the genitals, while evaluating the echographic parameters of the uterus, in particular the anterior-posterior size, length, width, and determination of the length, width, and thickness of the ovaries. According to ultrasound data, the size of the tumor was determined, and the thickness of the endometrium was measured. CT, MRI, and PET-CT examinations were also performed on the patients included in the study. In this study, a marker of carbohydrate metabolism, the F18-fluorodeoxyglucose radiopreparation, was used for PET. Indications for PET were: diagnosis of primary tumors of various locations, determining the prevalence of the process, the presence of metastases, planning radiation therapy and evaluating the effectiveness of antitumor therapy. PET studies were conducted at the center for Nuclear Medicine in Baku.

To study the informative value of hormone indicators in women with tumors of the reproductive system, indicators of FSH, LH, estradiol, estrone, DHEA-C, progesterone, testosterone and prolactin in the blood serum were determined. Hormonal studies were performed using enzyme immunoassay on the Roche Cobas E 411 device at the medical Plaza clinic in Baku. Blood biochemical parameters were also studied, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT), creatinine, fasting glucose, total protein, urea, uric acid, residual nitrogen, alkaline phosphatase, cholesterol, and triglycerides. All patients were identified indicators of the cancer marker CA-125.

The obtained quantitative and qualitative data were subjected to statistical processing by special methods of medical statistics, taking into account modern requirements. The methods of variational, correlation, variance, discriminant, and ROC analyses were applied. Methods of variational statistics are used to analyze the obtained quantitative digital data. The average values of the obtained samples (M), their standard deviations (σ), standard errors (m), 95% confidence intervals (95% CI), minimum (min) and maximum (max) values of the series are calculated. To estimate the difference between the variational series, the parametric t-student criterion was previously used.

Results of the study and their discussion. In the study, the incidence of endometrial cancer was 16.3% (n=50). The stages of diagnosis of endometrial cancer according to the FIGO classification are shown in table 1.

Table 1

Stages of endometrial cancer diagnosis by FIGO (based on a prospective study)

Stages of endometrial cancer	Number of patients (n=50)	
	Abs.	%
Stage I (tumor within the body of the uterus)	34	68
- IA (the tumor is limited to the endometrium, or invaded the myometrium by < ½ thickness)	21	42
- IB (the tumor is invaded in the myometrium by > ½ thickness)	13	26
Stage II (the tumor sprouts the stroma of the cervix, but does not extend beyond the uterus)	15	30
Stage III (metastases in pelvic lymph nodes)	1	2

In endometrial cancer in the postmenopausal period, 68% of patients have a tumor detected at stage I, 30% of patients-at stage II, and only 2% of patients at stage III of the tumor process. According to a retrospective study, the incidence of endometrial cancer in the postmenopausal period was 21.6%. In 86.2% of cases, endometrial cancer was diagnosed at the first stage of development of the tumor process.

The study found that the average age of patients with ovarian cancer was 59.1±0.5 years. The average age of patients with endometrial cancer was 62.4±1.0 years. In patients with endometrial hyperplastic processes, the age index ranged from 58.9±0.9 years, in patients with uterine fibroids from 55.1±0.2 years. The age of patients diagnosed with benign ovarian neoplasms was 62.3±1.1 years.

The study of the age factor in the Genesis of tumors of the reproductive system suggests that the postmenopausal period itself, against the background of significant neurohormonal changes in the female body, is a high risk factor for the development of neoplasms.

The age of ovarian cancer patients included in this study ranged from 48-77 years. There is a high risk of ovarian cancer in women over 55 years of age. The peak of endometrial carcinoma occurs at the age of 70–74 years, which allows us to consider the age of a woman and the duration of the postmenopausal period as high risk factors for the development of genital neoplasms.

Analysis of the results of a prospective study revealed that ovarian cancer in the postmenopausal period was diagnosed in 66% of cases at stage III of the tumor process, in 15.5% at stage I, and in 10.3% at stage IV of the tumor process. This is often due to the asymptomatic course of the disease or the presence of non-specific symptoms.

The study of the frequency of complaints in patients with ovarian cancer is presented in table 2.

Table 2

The frequency of complaints in patients with ovarian cancer in postmenopausal women

Complaints	Abs.	%
Pain	85	87.6
Spotting	2	2.1
Bleeding from the genital tract	2	2.1
The increase in the volume of the stomach	70	72.2
Fatigability	50	51.5
Dysuric phenomena	33	34.0
Difficulty breathing	19	19.6
Ascites	54	55.7
Constipations	3	3.1
Increased blood pressure	71	73.2
Sleep disturbance	78	80.4

As a result of the study, it was found that out of 97 patients with ovarian cancer, 18 (18.6%) patients with ovarian cancer had a combination of the underlying disease with other genital tumors, the frequency of which is shown in table 3.

Table 3

The rate of increase of ovarian cancer with other tumors of the genitalia in postmenopausal women

Concomitant tumors	Ovarian cancer (n=18)	
	Abs.	%
Hysteromyoma	13	72.2
Diffuse endometrial hyperplasia	3	16.7
Endometrial polyp	2	11.1

According to the results of this study, the frequency of combination of endometrial hyperplastic processes with other genital tumors was 35.4%. Endometrial hyperplastic processes are most often combined with uterine myoma, the frequency of which ranges from 72.7% to 100% in this group of patients. In postmenopausal women, the frequency of combination of uterine fibroids with concomitant genital tumors was determined in the range of 38.3%. Diffuse endometrial hyperplasia (83.3%), ovarian tumour-like formations (1.1%) and focal endometrial hyperplasia (5.5%) were most frequently detected in patients with uterine fibroids.

The study of the body mass index revealed that in patients with ovarian cancer, the body mass index was 28.7 ± 0.6 kg / m². The body mass index for endometrial cancer was in the range of 32.7 ± 1.0 kg / m². For benign ovarian neoplasms, the BMI of patients was within 27.3 ± 2.0 kg / m². BMI in patients with endometrial hyperplastic processes in the postmenopausal period was 31.9 ± 0.8 kg / m². Of 34 patients with diffuse endometrial hyperplasia without atypia, 20 (58.8±8.4%) had various degrees of obesity. The rate of grade I obesity was $29.4 \pm 7.8\%$ (n=10), grade II frequency was within $17.6 \pm 6.5\%$ (n=6), and grade III obesity rate was $11.8 \pm 5.8\%$ (n=4). In atypical endometrial hyperplasia, the frequency of obese patients was slightly higher and amounted to $66.7 \pm 12.5\%$ (n=10). In patients with endometrial polyp, this indicator was $68.8 \pm 11.6\%$ (n=11). In patients with progressive growth of myomatous nodes in the postmenopausal period included in our study, the BMI was 29.5 ± 0.9 kg / m² and ranged from 19 to 44 kg/m². The results obtained allowed us to determine that in patients with genital tumors in the postmenopausal period, there is an increase in BMI indicators, reflecting the presence of changes in body weight and obesity of varying severity. At the same time, the maximum BMI values were observed in patients with endometrial pathologies, including endometrial cancer. Obesity has been found to be a high risk factor for endometrial cancer in women over 30 years of age. An increase in BMI increases the likelihood of developing endometrial cancer by 5 times.

In patients with ovarian cancer, the features of menstrual, reproductive and generative functions were studied. It was found that the average age of menarche was 13.2 ± 0.2 years. At the same time, 77.3% of patients had regular menstruation, and 22.7% had irregular menstrual cycles. The study of the history of patients with ovarian cancer revealed a high frequency (29.2%) of artificial termination of pregnancy. It should be noted that 91.8% of patients had more than 4 pregnancies, of which 64.4% of pregnancies ended in term delivery.

The study showed that the menstrual, reproductive and generative functions of the examined patients did not directly affect the risk of ovarian cancer. At the same time, the high rate of artificial termination of pregnancy seems to be a risk factor for ovarian cancer. One in three patients with ovarian cancer had a history of induced abortions.

The study of the obtained data revealed a relatively high frequency of gynecological diseases in the reproductive period (16.5%). Among gynecological diseases, ovarian cysts prevailed, the frequency of which was 56.3%. This suggests that ovarian tumours are a risk factor for ovarian cancer. The structure of gynecological operations in the reproductive period in patients with ovarian cancer in postmenopause is shown in the fig. 1.

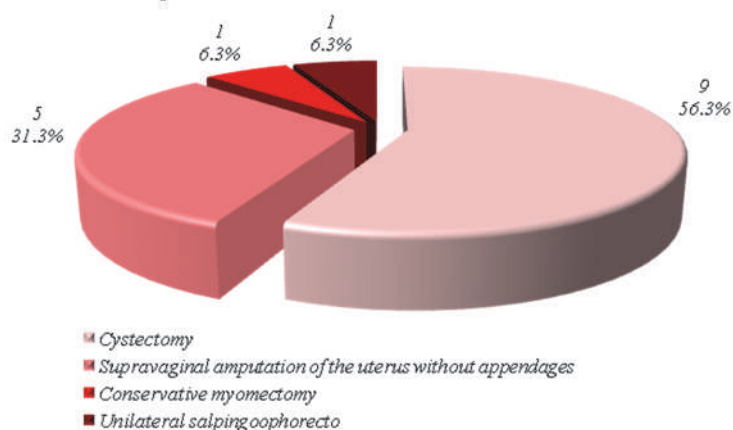


Fig. 1. The structure of gynecological surgery in the reproductive period in patients with ovarian cancer in postmenopausal women.

The study of the frequency of surgical interventions allowed us to establish that the frequency of surgical interventions for extragenital pathology in postmenopausal ovarian cancer patients was 18.6%.

The frequency of gynecological operations in the reproductive period in patients with ovarian cancer was 16.5%.

The results obtained allow us to state that the presence of tumor processes in the reproductive system and surgical interventions are an unfavorable background for the development of neoplastic processes in the postmenopausal period.

In postmenopausal patients with endometrial cancer, menstrual function was within the range of physiological fluctuations in the reproductive period. The study of the features of reproductive function allowed us to establish the presence of 4 or more pregnancies in 55.3% of patients. At the same time, in the anamnesis of these women, there was a high frequency of artificial termination of pregnancy, 46.8% of patients had a history of 2 or more abortions.

It should be noted that 14.9% of patients with endometrial cancer in the postmenopausal period had primary infertility, which was the reason for long-term hormone therapy.

Studies of reproductive function in patients with endometrial hyperplastic processes revealed that 85.5% of women had a history of high birth rate, of which 100% of women had artificial abortions. The frequency of infertility in this group of patients was 5.2%. There was no delivery in 14.5% of patients with pregnancy. It is important to have a history in patients with postmenopausal hyperplastic processes of episodes of recurrent endometrial hyperplasia in the reproductive period, which is a consequence of hormonal imbalance. This pathology of the reproductive period was observed in the anamnesis of every fifth patient from this group. A relationship was established between the number of pregnancies, childbirth and the risk of endometrial cancer in later life of women.

The relationship between a woman's age during the first pregnancy and the development of endometrial cancer was also established. Young pre-pregnant women are susceptible to developing endometrial cancer in later life.

Research conducted by the SGO (Society of Gynecological Oncology) working group on endometrial cancer has shown a steady increase in the incidence of endometrial cancer in older women. At the same time, the rate of diagnosis of endometrial cancer in the early stages is 75%. The average age of endometrial cancer diagnosis is 60 years. At the same time, it was found that the frequency of this pathology in people of Caucasian nationality significantly exceeds similar indicators in women of the African-American race [12]. Women who go through menopause after age 55 are at risk for developing ovarian, uterine, and breast cancer. The risk also increases with menarche before the age of 12. This is due to the fact that women who menstruate more than the average norm have a higher number of ovulations throughout their life and, consequently, are exposed to more estrogens. The greater the exposure to estrogens, the greater the risk of developing uterine and breast tumors; the greater the number of ovulations, the higher the risk of ovarian cancer [12, 13]

In recent years, according to scientific research, there is a clear relationship between body mass index, age, and in particular the development of endometrial cancer in women with long-term postmenopause. There was also an increased probability of ovarian cancer in unborn women, as well as in women with a history of artificial termination of pregnancy [5, 7]

Conclusion

It was found that risk factors for the development of neoplastic processes of genitalia in postmenopausal period are peri - and postmenopausal periods, the increase in body mass index, a longer duration of postmenopausal period, the presence of infertility in anamnesis, the presence of gynecological and endocrine diseases in the reproductive and perimenopausal periods, high frequency of induced abortion in anamnesis.

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Реферати

**ПОСТМЕНОПАУЗАЛЬНИЙ ПЕРІОД
ЯК ФАКТОР РИЗИКУ РОЗВИТКУ ПУХЛИН
ОРГАНІВ РЕПРОДУКТИВНОЇ СИСТЕМИ**

Гарашова М.А.

У дослідження було включено ретроспективний аналіз історій хвороби 301 жінки з різними пухлинами органів репродуктивної системи (Середній вік $61,6 \pm 0,4$ року). У проспективне дослідження було включено 306 жінок з доброякісними та злоякісними пухлинами геніталій в постменопаузальному періоді (Середній вік $59,3 \pm 0,4$ року). Всім хворим було проведено ехографію геніталій; вивчено рівні стероїдних гормонів і біохімічні показники крові; визначено показники онкомаркера СА-125. Було встановлено, що у хворих на рак яєчників індекс маси тіла склав $28,7 \pm 0,6$ кг / м², при раку ендометрія $32,7 \pm 1,0$ кг / м², при доброякісних новоутвореннях яєчників ІМТ був в межах $27,3 \pm 2,0$ кг / м², у хворих з гіперпластичними процесами ендометрія $31,9 \pm 0,8$ кг / м². У хворих на рак яєчників і рак ендометрія було виявлено високу частоту штучного переривання вагітності (29,2% і 46,8% відповідно). Таким чином, факторами ризику розвитку неопластичних процесів геніталій в постменопаузальному періоді є: збільшення індексу маси тіла, наявність безпліддя в анамнезі, наявність гінекологічних, ендокринних захворювань в репродуктивному і перименопаузальному періодах, висока частота штучного переривання вагітності в анамнезі.

Ключові слова: постменопауза, рак яєчників, рак ендометрія, гіперпластичні процеси ендометрія, індекс маси тіла.

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**ПОСТМЕНОПАУЗАЛЬНИЙ ПЕРІОД
КАК ФАКТОР РИСКА РАЗВИТИЯ ОПУХОЛЕЙ
ОРГАНОВ РЕПРОДУКТИВНОЙ СИСТЕМЫ**

Гарашова М.А.

В исследовании были включены ретроспективный анализ историй болезни 301 женщины с различными опухолями органов репродуктивной системы (Средний возраст $61,6 \pm 0,4$ года). В проспективное исследование были включены 306 женщин с доброкачественными и злокачественными опухолями гениталий в постменопаузальном периоде (Средний возраст $59,3 \pm 0,4$ года). Всем больным была проведена эхография гениталий; изучены уровни стероидных гормонов и биохимические показатели крови; определены показатели онкомаркера СА-125. Было установлено, что у больных с раком яичников индекс массы тела составил $28,7 \pm 0,6$ кг/м², при раке эндометрия $32,7 \pm 1,0$ кг/м², при доброкачественных новообразованиях яичников ИМТ был в пределах $27,3 \pm 2,0$ кг/м², у больных с гиперпластическими процессами эндометрия $31,9 \pm 0,8$ кг/м². У больных с раком яичников и раком эндометрия была выявлена высокая частота искусственного прерывания беременности (29,2% и 46,8% соответственно). Таким образом, факторами риска развития неопластических процессов гениталий в постменопаузальном периоде являются: увеличение индекса массы тела, наличие бесплодия в анамнезе, наличие гинекологических, эндокринных заболеваний в репродуктивном и перименопаузальном периодах, высокая частота прерывания беременности в анамнезе.

Ключевые слова: постменопаузальный период, рак яичников, рак эндометрия, гиперпластические процессы эндометрия, индекс массы тела.

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**METHODS OF RADIATION DIAGNOSTICS OF COMPLICATIONS IN COMBINED
CRANIOCEREBRAL TRAUMA AND ABDOMINAL TRAUMA**

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This article is devoted to one of the problems of medical radiation diagnostics of combined trauma. The specifics and features of instrumental monitoring of victims of combined craniocerebral and abdominal trauma were determined. The article presents data from a prospective survey of 142 victims of this type of injury. In some cases, patients with combined craniocerebral and abdominal injuries received x-rays of the abdominal organs. The number of victims surveyed was 42 (29,5%). Due to the difficulty of diagnosis associated with the condition of the victims and for more accurate research in the future, an ultrasound study was conducted for all. Carrying out the method of review radiography of the abdominal organs in victims with combined craniocerebral and abdominal injuries was difficult due to the condition of the patients. In case of combined cranio-abdominal trauma, ultrasound diagnostics and computer tomography examination of the brain and abdominal organs of the victims were performed. The sensitivity, specificity, and accuracy of these research methods were studied and proven, and their inclusion in the diagnostic algorithm was recommended.

Keywords: combined trauma, cranio-abdominal trauma, abdominal trauma, ultrasound, computed tomography,

The work is a fragment of a doctoral dissertation: "Prognostic value of modern methods of radiation diagnostics in severe combined injuries".

Injuries occupy one of the leading places in the structure of causes of death up to 40–45 years. The death rate from accidents and injuries is constantly growing and every year this increase is up to 1%. Despite the fact that only 8-10% of those admitted to hospital treatment are victims of combined trauma. the mortality rate for combined injuries is 70% [10, 12, 14].

Among the victims of injuries, 34% are abdominal injuries, ranking third in the structure of traumatic injuries after musculoskeletal and craniocerebral injuries [11, 13]. Among the combined injuries the most common is craniocerebral and abdominal trauma. A significant portion of abdominal injuries require surgery. Along with this, complications of the abdominal component in combined injuries are 7.8-40.8% [8, 11, 13]. The frequency and nature of abdominal complications vary depending on the duration of the injury. In the first two days, in the acute and initial period of injury, the most common complication is internal bleeding, which occurs in 2–3 cases (5%) [2] and peritonitis with the frequency of 1.3%. In later periods, in the second period of traumatic injury, on days 3–7, the most common complications are infections – 4–12%, intraperitoneal abscesses–2–4. 8%. [4], intestinal obstruction–1.3%. gastrointestinal bleeding–0.5-1%. The third and fourth periods of abdominal injury are characterized by the following injuries: intestinal fistulas –1.5-1.8%. intestinal obstruction –1.5–1.8%. internal abdominal abscesses–1.5% [11, 13]. In addition, the so-called abdominal component syndrome develops and the frequency of its occurrence in combined abdominal injuries is 5–8.2% [4, 10, 13].

Among the combined injuries, craniο–abdominal trauma is characterized by a peculiar pathogenetic complex of symptoms and syndromes. This symptom complex combines the features of both traumatic brain injury and damage to the abdominal cavity of the retroperitoneal space. Along with this, in case of combined craniocerebral and abdominal trauma, new isolated symptom complexes may be formed that are not typical for this type of injury [7, 8, 9].

A characteristic feature of craniο-abdominal trauma is the interrelated complications that occur with this type of damage. In this type of combined injury, the severity of the victim's condition is aggravated by the severity of the pathological condition associated with the traumatic lesion. On the other hand, if we consider separately craniocerebral and abdominal injuries, each of them is characterized by a severe course, various types of complications that aggravate together and separately and are the cause of the severe course of this type of injury. In addition, damage to the nervous system inevitably leads to a violation of the regulatory and coordinating functions of the autonomic nervous system, which in turn significantly reduces the effectiveness of compensatory mechanisms and increases the risk of complications. One of the characteristic features of combined craniocerebral and abdominal trauma is false dislocation syndrome. The severity of this syndrome depends on the severity of damage to the Central nervous system and is very closely related to the volume of hemoperitonium. A feature of combined craniocerebral and abdominal trauma is that when the parenchymal organs of the abdominal cavity are damaged and extensive blood loss occurs, in the amount of 1.5–2 liters or more, severity of the victim's condition and development of complications depend on the timely stabilization of hemodynamic parameters and replenishment of blood loss in the patient. Taking into account the peculiarities of combined craniο–abdominal trauma, it should be noted that their diagnosis and treatment present certain difficulties even in highly specialized hospitals [9, 12].

With combined craniocerebral and abdominal trauma, the severity of the condition and often absent consciousness of the victim lead to the inability to collect anamnestic data, clinical and diagnostic methods of examination are difficult or uninformative, and laboratory studies are not specific [1, 4]. All this makes it difficult to adequately assess the severity of the victim's condition to diagnose complications in time, which negatively affects the volume and timeliness of medical measures and, ultimately, is an unjustified cause of high mortality in this type of combined injury.

Taking into account the above, it is necessary to develop clinical research monitoring and an effective algorithm that allows timely implementation of both diagnostic and full treatment measures.

The purpose of the study was to determine the features and effectiveness of instrumental monitoring and develop a diagnostic algorithm for victims with combined traumatic brain and abdominal injuries.

Materials and methods. A prospective examination of 142 patients with combined craniocerebral and abdominal injuries who were admitted to the Clinical Medical Center of Baku was conducted. The criteria for selecting victims in the study group were as follows:

- the presence of combined craniocerebral and abdominal injuries.
- possibility of conducting an instrumental survey.
- no fatality of the victim within 24 hours.

In cases of combined craniocerebral and abdominal injuries, instrumental monitoring of the abdominal component was performed by diagnosing abdominal injuries using ultrasound and computed tomography (CT) examinations. Ultrasound was performed on a Toshiba X cario SSA–660A device (Toshiba, Japan) with a convex sensor – 3.75 Hs, a mechanical sector sensor – 2-5Hs in B–mode, as well as a portable ultrasound device Toshiba Nemio XG SSA–580A (Toshiba, Japan) with a linear sensor 3.5-

5.0 Hs. Computed tomography was performed on a Toshiba Aquilion 16 TSK-101 (Toshiba Corporation, Japan). The severity of mechanical damage in combined trauma was determined by the scale (AIS Abbreviated Injury Scale); the severity of the injury was determined by the ISS scale (Injury Severity Score); the level of consciousness of the victim was determined by the Qlazqo coma scale. In combined trauma, severe damage to the craniocerebral or abdominal components was determined on the AIS scale ≥ 3 ; if the injuries were not severe, they were evaluated on the AIS scale as AIS3.

The results of the study were subjected to statistical processing using the Microsoft Office (Excel) Statistika 6.0 package. the planning Standard was checked and the student's criterion (+) was calculated. The sensitivity, specificity, and accuracy of the study were calculated using generally accepted formulas.

Results of the study and their discussion. Of the total number of examined 142 people who received a combined traumatic brain and abdominal injury, the number of victims was 93 men (65.4%), the number of women was 49 (34.6%), respectively. The average age of the victims was 40.3 ± 2.0 years. The ISS score was 32.4 ± 12.7 points. When providing assistance to victims with combined craniocerebral and abdominal injuries, it is mandatory to conduct diagnostic studies and provide medical measures. And in general, when the victim is in a serious condition, diagnostic tests are carried out simultaneously with resuscitation measures.

In some cases, patients with combined craniocerebral and abdominal injuries received x-rays of the abdominal organs. The number of victims surveyed was 42 (29.5%). Due to the difficulty of diagnosis associated with the condition of the victims and for more accurate research in the future, an ultrasound study was conducted for all. Carrying out the method of review radiography of the abdominal organs in victims with combined craniocerebral and abdominal injuries was difficult due to the condition of the patients. The presence of pain and motor arousal of the victims made it difficult to perform diagnostic measures. It was not possible to conduct research in an upright position due to the severity of the victims' condition. Only 18 (42.8%) patients had abdominal x-rays that revealed injuries to internal organs and the presence of intra-abdominal bleeding. In other cases, the survey radiography did not allow to diagnose damage to the abdominal organs. To avoid diagnostic errors, as well as deterioration of the condition of victims with combined craniocerebral and abdominal trauma in the future, all 42 patients underwent ultrasound examination.

Thus, in the case of combined craniocerebral and abdominal trauma, the diagnosis of injuries to the abdominal organs during a review x-ray examination, the accuracy of diagnosis and information content was 43%.

All victims were required to have an ultrasound of the abdominal cavity and pleural sinuses, in addition to a CT scan of the brain. After admission to the hospital of the victim with a combined injury, an ultrasound study was conducted for 20 minutes. During the initial ultrasound examination of the abdominal cavity, no signs of internal bleeding were detected or these symptoms were doubtful. Taking this into account, during the first day, patients were dynamically monitored every 2-3 hours. Then, until the symptoms of damage to the abdominal organs were excluded, ultrasound was repeated 2-3 times a day. With extensive soft tissue emphysema, the use of ultrasound is limited. After 20 hours repeated ultrasound examination allowed to determine the presence of free fluid in the abdominal cavity. Ultrasound examination of 113 (79.5%) victims allowed making a correct diagnosis. Abdominal injuries and internal bleeding were correctly diagnosed in 61 (42.9%) victims with combined craniocerebral and abdominal injuries. In some cases ultrasound to diagnose abdominal injuries was difficult or even impossible. This was observed in 42 (29.5%) injured patients. Of these subcutaneous emphysema was found in 12 (8.9%) cases, intestinal paresis was found in 7 (4.9%) patients, restless behavior of the victim or urgent and urgent resuscitation was found in 8 (5.6%) cases, abdominal adhesions were detected in 2 (1.4%) patients.

In combined craniocerebral and abdominal injuries, ultrasound diagnostics of abdominal injuries was 77.3% sensitivity, 100% specificity, and 88.2% accuracy of diagnosis, respectively.

CT scans of the abdominal cavity and retroperitoneal space were performed in 32 (22.5%) injured patients. CT examination revealed liver rupture in 9 (28.1%) and intra-organ spleen hematoma in 4 (12.5%) patients. In 3 (9.3%) of the victims, a retroperitoneal hematoma was detected on CT. Diagnostic mistakes and errors were not detected during the CT study. The use of this diagnostic method of research allowed not only to detect the presence of free fluid in the abdominal cavity and hemoperitoneum, but also in all cases of examination, the topical diagnosis was correctly made. This research method is characterized by 100% sensitivity, specificity and accuracy in the diagnosis of patients with combined traumatic brain and abdominal trauma. But the use of CT diagnostics has certain difficulties. When performing CT, it is necessary to transport the victim from the intensive care unit to a specially equipped room and this transportation in turn is fraught with the risk of deterioration of the victim's condition.

Until recently, the diagnosis of craniocerebral disorders was considered a difficult task in cases of combined cranio-abdominal trauma and severe damage to the abdominal organs. Severe, having a significant impact on the outcome of combined neurotrauma is damage to the abdominal organs and the presence of internal bleeding, which is characterized by a high frequency of life-threatening complications and dependence on accuracy correct timely diagnosis and surgical treatment. In case of combined craniocerebral and abdominal trauma. CT examination is necessary for timely diagnosis of both abdominal injuries and thoracic trauma. The presence of intraperitoneal bleeding and the resulting hypoxia causes rapid development of brain edema. dislocation. and as a result. the victim's serious condition worsens even more. In addition. numerous fractures of the ribs and limbs are complicated by hemiparesis. which is a characteristic complication in internal hematoma or severe damage in traumatic brain injury. CT examination of the brain was performed in 38 (26.7%) victims with severe traumatic brain injury. In all cases the diagnostic study carried out in the end allowed us to correctly assess the severity of the lesion and. accordingly. choose and implement therapeutic measures. Taking into account the above, we can state with confidence that the sensitivity. Specificity, and accuracy of diagnosis in CT examination was 100%.

Thus, combined craniocerebral and abdominal trauma is characterized by a severe course and complex diagnostic features. As shown in our study, in the presence of combined craniocerebral and abdominal traumas, the patient's condition and the damage outcome in the first 3-4 days are determined mainly by the severity of damage to the abdominal organs, the degree of bleeding as well as the timeliness adequate diagnostic and therapeutic measures. At a later date, 6-10 days, the severity of the victim's condition is determined by brain damage and its complications. The use of instrumental examination methods is an important stage in the diagnosis of abdominal and brain injuries in combined cranio-abdominal trauma. In case of combined trauma. all victims in a restless state should immediately undergo an ultrasound examination upon admission to detect signs of internal bleeding or damage to the abdominal organs. If there are none, this diagnostic test should be repeated every 4 hours during the day.

The data of our study coincide with the indices by A. A. Konanenko and co-authors [2, 5]. In their studies, as well as in our own, it is stated that in the case of combined craniocerebral and abdominal trauma, ultrasound diagnostics is an important and exceptional method for detecting damage to the abdominal cavity. On the other hand, with extensive soft tissue emphysema, the diagnostic capabilities of this method are limited.

It should be noted that combined traumatic brain injury is characterized by a high rate of complications and mortality, which ranges from 25 to 65%. The complexity of diagnosis in this type of combined trauma is determined by severity of the victim's condition, lack of consciousness. as well as various types of damage to parenchymal and hollow organs and systems [3,10,14]. Against the background of the patient's severe condition, traumatic shock, severe craniocerebral damage in combined trauma, damage to the abdominal cavity or retroperitoneal space generally remains undiagnosed.

Many hospitals and emergency departments have their own specific criteria for providing medical care to victims with combined cranio-abdominal trauma, which in turn negates diagnostic measures [6]. Based on the performed studies and the results obtained, we propose to use the following algorithm of diagnostic methods in patients with combined craniocerebral and abdominal trauma. The diagnostic algorithm should include both ultrasound examination and CT examination for the first 20-30 minutes of admission of the victim to the hospital, if there are no indications for surgical intervention, the results of diagnostic research methods should be repeated in the next 2-6 hours.

In the presence and confirmation of damage to the abdominal organs and intra-abdominal bleeding, immediate surgical intervention is necessary.

During early hospitalization (in the first hours after the injury), there are often no obvious clinical signs of intraabdominal injuries. Abdominal trauma in these patients can be suspected due to the presence of unstable hemodynamics in the absence of signs of external bleeding. However, interpretation of these data is difficult for victims with impaired consciousness as a result of severe traumatic brain injury.

Thus. combined craniocerebral and abdominal trauma is characterized by the severity of diagnosis and timely diagnosis. Timely diagnosis of abdominal injuries and assessment of intra-abdominal bleeding depends on the adequacy of instrumental methods of examination conducted by the victim.

Thus, in the case of combined craniocerebral and abdominal trauma in order to detect internal bleeding and damage to the abdominal organs, the ultrasound diagnostic method is characterized by 100% specificity, 77.3% sensitivity, and 88.2% accuracy. CT examination is determined by specificity, sensitivity and accuracy up to 100%.

Conclusion

Currently, in this type of combined injury, ultrasound and CT should be included in the methods of investigation of injuries to the abdominal cavity and brain for early, timely diagnosis, as well as for the prevention of various types of complications.

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Реферати

**МЕТОДИ ПРОМЕНЕВОЇ ДІАГНОСТИКИ
УСКЛАДНЕНЬ ПОЄДНАНОЇ ЧЕРЕПНО-
МОЗКОВОЇ І АБДОМІНАЛЬНОЇ ТРАВМИ
Гасимзаде Г.Ш.**

Дану статтю присвячено одній з проблем охорони здоров'я - променевої діагностиці поєднаної травми. Визначено специфіку і особливості інструментального моніторингу постраждалих від поєднаної черепно-мозкової і абдомінальної травм. У статті представлено дані проспективного обстеження 142 постраждалих даним видом травми. Постраждалим з поєднаною черепно-мозковою та абдомінальною травмою в ряді випадків проводилась рентгенографія органів черевної порожнини. Кількість обстежуваних потерпілих склало 42 (29,5%) особи. У зв'язку з ускладненням діагностики, пов'язаним зі станом потерпілих і для більшої точності дослідження в подальшому всім було проведено УЗ дослідження. Проведення методу оглядової рентгенографії органів черевної порожнини у потерпілих з поєднаною черепно-мозковою та абдомінальною травмою було утруднене у зв'язку зі станом хворих. За поєднаної краніо-абдомінальної травми проведено ультразвукову діагностику і комп'ютерно-томографічне дослідження головного мозку і органів черевної порожнини постраждалим. Було вивчено і доведено чутливість, специфічність і точність цих методик досліджень та рекомендовано включення їх в алгоритм діагностики.

Ключові слова: поєднана травма, черепно-мозкова, абдомінальна травма, ультразвукове дослідження, комп'ютерно-томографічна діагностика.

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**МЕТОДЫ ЛУЧЕВОЙ ДИАГНОСТИКИ
ОСЛОЖНЕНИЙ СОЧЕТАННОЙ ЧЕРЕПНО-
МОЗГОВОЙ И АБДОМИНАЛЬНОЙ ТРАВМЫ
Гасымзаде Г.Ш.**

Данная статья посвящена одной из проблем здравоохранения - лучевой диагностике сочетанной травмы. Определены специфика и особенность инструментального мониторинга пострадавших от сочетанной черепно-мозговой и абдоминальной травм. В статье представлены данные проспективного обследования 142 пострадавших данным видом травмы, Пострадавшим с сочетанной черепно-мозговой и абдоминальной травмой в ряде случаев проводилась рентгенография органов брюшной полости. Количество обследуемых потерпевших составило 42 (29,5%) человек. В связи с затруднением диагностики, связанное с состоянием потерпевших и для более точности исследования в дальнейшем всем было проведено УЗ исследование. Проведение метода обзорной рентгенографии органов брюшной полости у потерпевших с сочетанной черепно-мозговой и абдоминальной травмой было затруднено в связи с состоянием больных. При сочетанной кранио-абдоминальной травме проведена ультразвуковая диагностика и компьютерно-томографическое исследование головного мозга и органов брюшной полости пострадавшим. Были изучены и доказаны чувствительность, специфичность и точность этих методик исследований и рекомендовано включение их в алгоритм диагностики,

Ключевые слова: сочетанная травма, черепно-мозговая, абдоминальная травма, ультразвуковое исследование, компьютерно-томографическая диагностика.

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PRECONDITIONS FOR EARLY AND LATE INTRAVENTRICULAR HEMORRHAGES IN PRETERM VERY LOW BIRTH WEIGHT INFANTS

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The risk factors for early and late intraventricular hemorrhages (IVH) in very preterm newborns were investigated in a cohort study. One hundred eleven infants with a mean gestation age of 29.59 ± 2.59 wks. were under observation. The risk factors connected to the development of early and late hemorrhages were different. Opposite to the early IVH, development of the late hemorrhages was neither dependent on birth weight and gestation age, nor on infectious pathology of perinatal period. The relevant risk was determined by infant's condition at birth and arterial hypotension that developed within the first 24 hrs. of newborn's hospitalization. Development of severe respiratory distress syndrome (RDS) was reliably connected with IVH independently on infant's age. According to the results of logistic regression analysis, the risk of early IVH was significantly associated with RDS severity (odds ratio [OR]: 6.05; 95% confidence interval [CI]: 1.35–27.17), and the probability of late IVH was significantly influenced by arterial hypotension within the first 24 hrs. of hospitalization (OR: 16.6; 95% CI: 2.69–102.43).

Keywords: intraventricular hemorrhages, age of occurrence, risk factors, prevention, very preterm infants.

The work is a fragment of the research project "Improving methods of diagnosis, treatment and prevention of the most common diseases of childhood", state registration No. 0117U001083.

Intraventricular hemorrhage (IVH) often complicates the neonatal period in very preterm (gestational age <32 weeks) infants and represents one of the most severe perinatal brain lesions. Its development is associated with high mortality and may be an important independent cause of long-term neurological and cognitive impairment in surviving infants [9, 11].

Approximately half of the cases of hemorrhage in infants with very low birth weight occur during the first 6 hours of life, and 38% – after 24 hours of life [1]. IVH is considered early if detected within the first 72 h of life and late if diagnosed after 72 h [15].

The high prevalence of IVH in the group of least mature newborns, the association of this complication with high mortality, and the threat of severe long-term consequences and disability determine the importance of studying the risk factors for IVH and possible mechanisms of their occurrence.

The purpose of the study was to determine the risk factors for early and late IVH in the modern cohort of very preterm infants.

Materials and methods. A cohort of 111 very preterm infants with birth weight less than 1.500 g was under observation in specialized neonatology departments of the Lviv Regional Clinical Hospital (LRCH). The inclusion criteria were hospitalization within the first 3 days of life and the absence of significant malformations.

IVH were diagnosed using cranial ultrasound (CUS), which was performed on the third day of life. To assess the evolution of existing hemorrhages and diagnose late IVH, according to the developed protocol, further examinations were performed on 5-7, 9-11, 14-16 and 24-28 days of life, as well as at postmenstrual age of 36 weeks or before discharge from the hospital. CUS was also performed regardless of the infant's age if there were clinical signs indicating the possible development of IVH. Digitae Sonoace 5500 ultrasound machine (Medison, South Korea) and 5–7.5 MHz convex sensors were used. The severity of the hemorrhages was assessed using the Papille classification.

All children were distributed into 2 groups depending on the presence of hemorrhage. The main group included 21 (19%) infants with IVH diagnosed within 3 days of life, and the control group consisted of 90 (81%) newborns in whom no hemorrhage was detected at this age. Late hemorrhage developed in 17 (15%) infants from the latter group, these babies were included into the main subgroup, and 73 (66%) newborns, who did not have this CNS pathology before discharge from the hospital, were attributed to the control subgroup.

The obtained data were analyzed using standard descriptive statistics methods, categorical, and multivariate logistic regression analysis. Categorical data are presented as numbers (%). The measurements with normal distribution are presented as means (\pm standard deviations). Nonparametric data are presented as medians [interquartile ranges]. Parametric continuous measurements were compared using t-test and medians were compared with the Mann–Whitney U test for non-parametric data. The chi-squared test was applied to categorical data. A *p* value less than 0.05 was considered significant.

Results of the study and their discussion. The mean gestational age of infants in the study was 29.59 ± 2.59 weeks. Seventeen infants (15%) had birth weight less than 1000 g, 1000–1249 g – 32 (29%) and 1250–1500 g – 62 newborns (56%). The mean age of babies in the main group at the time of hospitalization was 8 [3–19] h versus 10.5 [4–25] h for infants in the control group ($p=0.51$).

Newborns with early hemorrhage had a lower gestational age (28.048 ± 1.91 weeks vs. 29.94 ± 2.61 weeks in the control group; $p=0.002$) and birth weight (respectively 1147.62 ± 236.81 g vs. 1255.22 ± 236.004 g; $p=0.063$). At the same time, these indicators did not differ significantly in infants from the main and control subgroups (respectively 28.82 ± 1.88 weeks vs. 29.72 ± 2.69 weeks; $p=0.19$; and 1204.12 ± 284.65 g vs. 1240.43 ± 230.95 g; $p=0.57$). The main group also differed from the control by a significantly larger proportion of infants with weight less than 1250 g (15 (71.4%) vs. 34 (37.7%); $p=0.014$). Instead, late hemorrhages were more common in bigger children.

Early IVH were significantly more often severe than late ones ($p<0.05$) (Fig. 1), and were more likely to cause death of infants accordingly (9 (42.9%) cases in the main versus 6 (6.7%) in the control group; $p=0.001$).

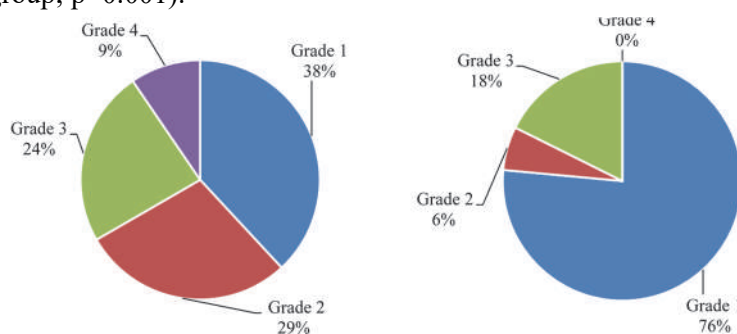


Fig. 1. Comparative distribution of early and late IVH by severity ($p<0.05$).

A comparative analysis of medical and social risk factors showed that mothers of infants with early IVH were more likely to be under 17 years of age (respectively 3 (14.3%) vs. 3 (3.3%) cases; $p=0.046$). At the same time, the majority of other risk factors in mothers of infants with IVH were not associated with either type of the hemorrhage. Only the frequency of stillbirths was significantly higher in

mothers whose children had late IVH (respectively 3 (17.6%) vs. 1 (1.4%) cases; $p=0.003$).

Antenatal steroid prophylaxis most significantly reduced the risk of early hemorrhages. Hemorrhages within the first 72 hours of life were less likely to develop in infants with intrauterine growth retardation. Instead, early IVH were significantly more often diagnosed in babies from mothers with bleeding in the first half of pregnancy or preeclampsia (table 1).

Table 1

Comparative frequency of pregnancy and delivery complications in groups and subgroups

Risk factors	Groups		<i>p</i>	Subgroups		<i>p</i>
	Main (n=21)	Control (n=90)		Main (n=17)	Control (n=73)	
Preeclampsia	11 (52.4)	24 (26.7)	0.035	3 (17.6)	21 (28.8)	0.62
Bleeding in the first half of pregnancy	7 (33.3)	9 (10.0)	0.006	3 (17.7)	6 (8.2)	0.26
Bleeding in the last trimester of pregnancy	7 (33.3)	10 (11.1)	0.098	0 (0)	10 (13.7)	0.16
Cesarean section	1 (4.8)	17 (18.9)	0.13	0 (0)	1 (1.4)	0.63
Rapid delivery	3 (14.3)	30 (33.3)	0.087	2 (11.8)	15 (20.5)	0.46
Duration of delivery > 24 h	5 (23.8)	16 (17.8)	0.55	4 (23.5)	16 (21.9)	0.91
Placental abruption	5 (23.8)	11 (12.2)	0.18	1 (5.9)	10 (13.7)	0.38
IUGR	0 (0)	15 (16.7)	0.052	1 (5.9)	14 (19.2)	0.22
Multiple pregnancy	3 (14.3)	16 (17.9)	0.71	3 (17.7)	13 (17.8)	0.99
Antenatal steroids	6 (28.6)	63 (70.0)	0.001	14 (82.3)	49 (67.1)	0.21

Notes: number of cases (%). IUGR—intrauterine growth retardation.

The risk of early IVH increased in case of chorioamnionitis (respectively 6 (28.6%) vs. 4 (4.4%) cases; $p=0.001$), fever during labor (respectively 3 (14.3%) vs. 1 (1.1%) case; $p=0.003$), or stained amniotic fluid (respectively 13 (61.9%) vs. 17 (18.9%) cases; $p=0.001$), however, effective antibacterial therapy reduced this risk (2 (9.5%) versus 36 (40.0%) cases respectively; $p=0.009$). At the same time, no significant associations have been established between infectious diseases in mothers and the occurrence of late IVH in their children. Early hemorrhages were significantly more common in infants with hypothermia, severe RDS, pneumothorax, early sepsis, clinical signs of patent ductus arteriosus (PDA), as well as in those who required controlled ventilation or early administration of dobutamine (table 2).

On the first day of hospitalization, infants with early hemorrhages were ventilated with significantly higher mean airway pressure (11.14±2.57 cm Hg vs. 9.064±1.89 cm Hg; $p=0.002$). They also had a lower pH (7.19±0.083 vs. 7.33±0.082; $p=0.001$) and higher partial pressures of CO₂ (paCO₂) (48 [38.3–54] cm Hg vs. 33 [28.4–38.9] mm Hg; $p=0.001$).

Resuscitation at birth with epinephrine and (or) chest compressions, hypotension with dopamine administration, severe RDS, pneumothorax, surfactant administration and use of controlled mechanical ventilation were associated with higher incidence of late IVH (table 2).

According to the results of LRA, occurrence of early hemorrhages was significantly associated only with the severity of RDS (odds ratio (OR) – 6.05; 95% confidence interval (CI): 1.35–27.17), hypotension within the first 24 hours of hospitalization reliably and independently affected the probability of late hemorrhage occurrence (OR–16.6; 95% CI: 2.69–102.43).

Table 2

Early neonatal period data in groups and subgroups¹

Risk factors	Groups		<i>p</i>	Subgroups		<i>p</i>
	Main (n=21)	Control (n=90)		Main (n=17)	Control (n=73)	
Intubation and MV	6 (28.6)	29 (32.2)	0.79	7 (41.2)	22 (30.1)	0.34
Chest compressions	3 (14.3)	4 (4.4)	0.1	3 (17.6)	1 (1.4)	0.003
Epinephrine administration	3 (14.3)	4 (4.4)	0.1	3 (17.6)	1 (1.4)	0.003
Apgar<4 at 5 min	4 (19.0)	9 (10)	0.25	1 (5.9)	8 (10.9)	0.53
MV at birth	14 (66.7)	44 (48.9)	0.16	11 (64.7)	33 (45.2)	0.17
CPAP at birth	1 (4.8)	13 (14.4)	0.23	1 (5.9)	12 (16.4)	0.26
Hypothermia at the time of hospitalization (<36.6°C)	20 (95.2)	6 (6.7)	0.001	0 (0)	6 (8.2)	0.22
Arterial hypotension ²	7 (33.3)	15 (16.7)	0.091	12(70.6)	3(4.1)	0.001
Dobutamine ²	4 (19.1)	1 (1.1)	0.001	0 (0)	1 (1.4)	0.63
Dopamine ²	6 (28.6)	14 (15.6)	0.17	12 (70.6)	2 (2.7)	0.001
Hypotension / dopamine at the age of >3 days	0 (0)	0 (0)	–	2 (11.8)	0 (0)	0.003
Severe RDS	14 (66.7)	29 (32.2)	0.004	17 (100)	11 (15.1)	0.001
Initial controlled MV	11 (52.4)	24 (26.7)	0.024	8 (47.1)	16 (21.9)	0.04
Surfactant therapy	8 (38.1)	18 (20.0)	0.084	7 (41.2)	11 (15.1)	0.02
Pneumothorax at the age of 1–3 days	1 (4.8)	0 (0)	0.04	0 (0)	0 (0)	–
Pneumothorax at the age of >3 days	0 (0)	9 (10)	0.13	9 (52.9)	0 (0)	0.001
Clinical signs of PDA at the age of 1–3 days	6 (28.6)	3 (3.3)	0.001	1 (5,9)	2 (2.7)	0.53
Early onset sepsis	13 (61.9)	2 (2.2)	0.001	0 (0)	2 (2.7)	0.49

Notes: 1–number of cases (%); 2–the first 24 hours of hospitalization; MV–mechanical ventilation; RDS – respiratory distress syndrome; PDA– patent ductus arteriosus; CPAP– continuous positive airway pressure.

In this study, we found that infants who developed IVH within the first 72 hours of life were more likely to be born to mothers whose pregnancies were complicated by preeclampsia and bleeding in the first half of pregnancy. Intrauterine growth retardation reduced the risk of this complication (table 1). Because preeclampsia was much more common in mothers of infants with early hemorrhages, and the incidence of intrauterine growth retardation was higher in babies without IVH, it could be thought that only severe or prolonged preeclampsia reduced the risk of IVH.

Our results confirm the known fact of the prophylactic effect of antenatal steroids on the occurrence of early IVH [14] but indicate a lack of such a protective effect against the late hemorrhages (table 1). The incidence of the latter type of IVH did not depend on the complicated course of pregnancy and childbirth (table 1) but was associated with the condition of infant at birth (table 2).

A comparative analysis of the frequency of infectious risk factors revealed a significant association between the presence of an infectious process in the mother's body before and during childbirth and the occurrence of early IVH. Studies by other authors have also established an association between chorioamnionitis and a higher incidence of IVH [8, 9]. Our results suggest that effective maternal antibacterial therapy is likely to reduce the risk of neonatal hemorrhage, as confirmed by other authors [3]. At the same time, we did not prove the effect of infectious diseases in mothers on the risk of late IVH in their infants.

According to the literature, birth asphyxia, and the use of resuscitation measures in the delivery room, as well as the need for mechanical ventilation immediately after birth are associated with a high risk of early IVH [7, 9]. Infants in the main group were more likely to require resuscitation after birth and had lower 5 min Apgar scores, but differences in the frequency of resuscitation interventions were not significant. In contrast, infants who required extended or complete resuscitation, were much more likely to develop late IVH (table 2).

In our study, children who did not have early IVH, were significantly more likely to be born by elective or urgent cesarean section, although no statistically significant differences were found between the groups. The elective cesarean section may have advantages in the prevention of IVH in newborns with gestational age of <30 weeks compared not only to vaginal delivery but also to the emergency cesarean section [6]. Meta-analysis data, which took into account all cases of cesarean section, showed its advantage in the prevention of IVH in premature infants with gestational age of <28 weeks, who required active resuscitation after birth. Should cases of elective cesarean section be the only ones considered, the results could have been better, as the need for an emergency cesarean section could be determined by conditions that increase the risk of IVH [4].

Treatment with constant positive airway pressure (CPAP) or mechanical ventilation did not significantly increase the risk of IVH occurrence within the first 72 hours of life, although initial controlled ventilation was a significant risk factor for both types of hemorrhage. Infants with early IVH were ventilated with significantly higher mean airway pressures during the first day of hospitalization, consequently, a larger tidal volume was used increasing the risk of IVH development [10]. Such infants were also significantly more likely to have acidosis and higher levels of p_aCO₂, which were connected to the occurrence of IVH by other authors [8].

The early IVH occurred much more often in younger and more immature infants with hypothermia at hospitalization, severe RDS, pneumothorax, sepsis, and clinical signs of PDA (Table 2). At the same time, we could not objectively assess the association between the onset of the early IVH and PDA because the routine echocardiographic examination was not possible during the study. Dobutamine administration was also associated with a higher risk of early IVH.

The risk of late IVH was significantly associated with primary resuscitation, which included epinephrine administration and/or chest compressions, hypotension, and its treatment with dopamine, severe RDS or pneumothorax, controlled mechanical ventilation, and surfactant treatment (table 2). The occurrence of IVH has been associated with the therapeutic use of surfactant by other authors [2], which indicates the reality of such an association in our settings. Significant associations between the development of IVH and hypotension, the use of inotropic drugs [8, 12], the presence of RDS [8], and its complications [13] have also been established in other studies.

Thus, the risk factors of early and late IVH differ, which confirms the idea of different mechanisms of their occurrence. Our data suggest a significant association between the severity of the condition of the premature infant, development of RDS, peculiarities of its course, and effectiveness of treatment with the occurrence of IVH not only within the first days after birth but also during the late neonatal period. This emphasizes the importance of adequate obstetric care, rational use of surfactant, and physiological respiratory support immediately after birth, including the use of CPAP, which can prevent the development of severe RDS and reduce the need for surfactant treatment [5, 14].

Conclusions

1. The severity of RDS in a premature infant is the main factor that significantly and independently determines the risk of IVH within the first 72 hours of life. Additional factors that increase this risk are complicated course of RDS, using asynchronous mechanical ventilation with rigid parameters, inotropes administration, mother's or infant's infection diseases (complications), and acidosis development. The incidence of early hemorrhages can be reduced with antenatal steroids administration. The onset of early IVH significantly increases the risk of infant death.

2. Late IVH, which occurs beyond 72 hours of life, is a common problem of very premature infants. The occurrence of this complication is significantly associated with impaired hemodynamics in the early postnatal period, increasing severity and complicated course of RDS, as well as methods of RDS treatment.

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Реферати

ПЕРЕДУМОВИ ВИНИКНЕННЯ РАННІХ ТА ПІЗНІХ ВНУТРІШНЬОШЛУНОЧКОВИХ КРОВОВИЛИВІВ У НЕДОНОШЕНИХ НОВОНАРОДЖЕНИХ З ДУЖЕ МАЛОЮ МАСОЮ ТІЛА

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У когортному дослідженні вивчені чинники ризику виникнення ранніх та пізніх внутрішньошлуночкових крововиливів (ВШК) у значно недоношених новонароджених. Під спостереженням перебували 111 немовлят із середнім гестаційним віком $29,59 \pm 2,59$ тиж. Установлено відмінність між чинниками ризику, що пов'язані з розвитком ранніх і пізніх крововиливів. На відміну від ранніх ВШК, імовірність виникнення пізніх крововиливів не залежала від маси тіла і гестаційної зрілості дитини, а також інфекційної патології перинатального періоду. Натомість, відповідний ризик визначався важкістю стану немовляти після народження і наявністю артеріальної гіпотензії в першу добу госпіталізації. Розвиток важкого респіраторного дистрес-синдрому (РДС) був вірогідно пов'язаний з виникненням ВШК незалежно від віку дитини. За підсумками логістичного регресійного аналізу ризик виникнення ранніх крововиливів достовірно визначався важкістю РДС (коефіцієнт співвідношення шансів (КСШ) – 6,05; 95% довірчий інтервал (ДІ): 1,35–27,17), а на ймовірність розвитку пізніх ВШК вірогідно впливала артеріальна гіпотензія в перші 24 год госпіталізації (КСШ – 16,6; 95% ДІ: 2,69–102,43).

Ключові слова: внутрішньошлуночкові крововиливи, вік на момент виникнення, чинники ризику, профілактика, значно недоношені новонароджені.

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ПРЕДПОСЫЛКИ ВОЗНИКНОВЕНИЯ РАННИХ И ПОЗДНИХ ВНУТРИЖЕЛУДОЧКОВЫХ КРОВОИЗЛИЯНИЙ У НЕДОНОШЕННЫХ НОВОРОЖДЕННЫХ С ОЧЕНЬ МАЛОЙ МАССОЙ ТЕЛА

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В когортном исследовании изучены факторы риска возникновения внутрижелудочковых кровоизлияний (ВЖК) у глубоко недоношенных новорожденных. Под наблюдением находились 111 младенцев со средним гестационным возрастом $29,59 \pm 2,59$ недель. Установлено отличие факторов риска, связанных с развитием ранних и поздних кровоизлияний. В отличие от ранних ВЖК, вероятность возникновения поздних кровоизлияний не зависела от массы тела и гестационной зрелости ребенка, а также от инфекционной патологии перинатального периода. Соответствующий риск определялся тяжестью состояния младенца после рождения и наличием артериальной гипотензии в первые сутки госпитализации. Развитие тяжелого респираторного дистрес-синдрома (РДС) было достоверно связано с возникновением ВЖК независимо от возраста ребенка. По итогам логистического регрессионного анализа риск возникновения ранних кровоизлияний достоверно зависел от тяжести РДС (коэффициент соотношения шансов (КСШ) – 6,05; 95% доверительный интервал (ДИ): 1,35–27,17), а на вероятность развития поздних ВЖК существенно влияла артериальная гипотензия в первые 24 часа госпитализации (КСШ – 16,6; 95% ДІ: 2,69–102,43).

Ключевые слова: внутрижелудочковые кровоизлияния, возраст на момент возникновения, факторы риска, профилактика, глубоко недоношенные новорожденные.

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ROLE OF SINGLE NUCLEOTIDE GENE POLYMORPHISMS IN THE DEVELOPMENT OF ULCERATIVE COLITIS

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The purpose of the work was to study the features of the IL1 (T-31C), IL1 (T-511C), IL6 (C-174 G), IL10 (592C> A), IL10 (C-819T), IL10 (G- 1082A), Tlr2 (Thr399ile), Tlr4 (Thr399ile), Tlr4 (Asp299Gly) genes polymorphic variants influence on the development of ulcerative colitis in patients. The total of 53 patients with ulcerative colitis were examined, the control group included a random sample of 49 healthy persons. Association of the ulcerative colitis development with the incidence of single nucleotide polymorphism of IL10 wild genotype gene (rs1800896), homozygous G/G genotype of single nucleotide polymorphism of the Tlr4 gene (rs4986790), the frequency of the T allele in the IL1 gene (rs1143627), C allele in the IL1 gene (rs 16944) and IL10 (rs1800872), which contributed to the disturbance and imbalance in the production of pleiotropic cytokines IL1 and IL10, predisposing to the development of ulcerative colitis and aggravating the course of the disease. The work shows associative links for single nucleotide polymorphism of Tlr4 gene (rs4986791) with the development of nonspecific ulcerative colitis both according to the multiplicative model with the C allele and according to the general inheritance model with the wild type of C/C genotype, which confirms the importance of this single nucleotide polymorphism in the development of the disease.

Key words: nonspecific ulcerative colitis, single nucleotide gene polymorphism, allele, genotype.

The work is a fragment of the research project "Features of the course, prognosis and treatment of comorbid conditions in diseases of the internal organs, with account of genetic, age and gender aspects", state registration No. 0118U004461.

Despite the advances in modern medicine, inflammatory bowel diseases (IBD) remain an unsolved problem. The prevalence as well as the incidence of IBD in different regions of the world has a wide range enough. The incidence of nonspecific ulcerative colitis (NUC) is detected in 1.25 - 20.3 new cases per 100 thousand of inhabitants, the prevalence is 21-268 cases per 100 thousand of inhabitants [2]. Crohn's disease (CD) is much less common and is detected in 2-4 cases per 100 thousand of inhabitants, the prevalence is 10-150 per 100 thousand of population [3].

Today IBD belong to the group of multifactorial diseases, often with a comorbid course, which development is effected by combined and modifying impact of genetic and environmental factors [1]. Today, there is evidence of the influence of 163 gene polymorphisms on the IBD development [8], which are primarily responsible for the integrity of the intestinal mucosa epithelium and the body's timely immune response to the influence of the external environment. In favor of a genetic predisposition to the IBD development, there are registered family cases, which range from 6 to 30%, and the same type of changes in 122 identical genes, identified in the study of epithelial cells of the intestinal mucosa, according to the data of the Kiel University (Germany) [4].

Speaking about the importance of genetic predisposition in the development of IBD [11], most frequently we talk about single nucleotide gene polymorphisms (SNPs), which can affect the rate of gene transcription, change the binding of transcription factors, mRNA level and its stability [9], affecting the immune response. Signal pattern-recognition receptors, as well as possible single nucleotide polymorphisms of genes responsible for the work of these structures, have a direct impact on the development of IBD. Thus, the researchers confirmed the influence of the SNP NOD2 / CARD15 gene (2104 C / T, 2722 G / C, G908R, Leu3020fsinsC) on the development of Crohn disease (CD), which is responsible for the recognition of intracellular pathogens that regulate inflammatory reactions in activation of nuclear factor NF- κ B [6].

Many authors have identified the correlation between the development of both NUC and CD and SNPs of toll-like receptors genes (TLr) [6, 7], which play a major role in the genetic predisposition to changes in bacterial colonization. Thus, the correlation of IBD with SNP of TLr 2 and TLr 4 genes was revealed. SNP of the TLr 4 gene Asp299Gly changes the resistance of the microorganism to gram-negative bacteria, thereby contributing to the onset of dysbiosis, which is often associated with IBD. The occurrence of SNP in the TLr 2 gene alters the susceptibility of the intestinal mucosa epithelium to infectious agents. The emergence of SNP Arg753Gln in the TLr 2 gene raises the propensity to develop a complicated course of infections.

In the development of IBD, the role of SNP genes has been proven in secreting cytokines [5, 10, 12], which provide local interactions of the immune system cells with specific cellular receptors. In the

presence of genetic changes responsible for the secretion of cytokines such as TNF - α , IFN - γ , transforming growth factor (TGF - β), IL 1 β , IL 5, IL 6, IL 8, IL 10, IL 13, IL 17, IL 22, the structure of the epithelial barrier may change. In future, such disorders can contribute to the penetration of pathogenic microorganisms into epithelial cells and cause the onset of inflammatory or proliferative changes in the intestine.

The purpose of the work was to study the features of some single nucleotide gene polymorphisms correlation with the development of nonspecific ulcerative colitis.

Materials and methods. In order to assess the possible effect of SNP on predisposition to the development of NUC, the detection frequency of genotypes and alleles of SNP genes IL1 (T-31C), IL1 (T-511C), IL6 (C-174 G), IL10 (592C> A), IL10 (C-819T), IL10 (G-1082A) Tlr2 (Thr399ile), Tlr4 (Thr399ile), Tlr4 (Asp299Gly) was studied in 53 patients with NUC treated at the Poltava N.V. Sklifosovsky Regional Clinical Hospital. The control group included a random sample of 49 healthy individuals. The mean age of patients was (35.4 \pm 3.9) years, the control group's mean age was (31.8 \pm 4.1) years. Among patients with IBD, men accounted for 28 (52.8%) patients, women - 25 (47.2%) patients, the onset of the disease was most frequently detected at the age of 18-43 years - 39 (73.6%) patients, the duration of the disease more frequently ranged from 5 to 10 years - 24 (45.3%) patients. The diagnosis was confirmed clinically, instrumentally, morphologically. The examination revealed a reliably more frequent lesion in the colon's left part - 31 (58.5%) patients, a mild degree of process activity was found in 25 (47.2%), moderate in 22 (41.5%) patients, severe disease activity - 6 (11.3%) patients.

To isolate SNPs in the human genome, the polymerase chain reaction (PCR) technique was used. By means of phenol-chloroform extraction method DNA was isolated, which was subsequently washed with 70% ethanol solution. After drying in air, further dissolution was carried out in deionized water, storage took place at a temperature of -20° C with a Rearch PCR Thermal Cycler (Corbett, Australia), using "Litech" genotyping kits (Russia) according to the instructions, amplification of the sequences was carried out using PCR.

To analyze the obtained amplification data, electrophoresis in 2% agarose gel was used, which was stained with ethidium bromide; a UV transilluminator was used for scanning. Genotyping was performed in the Laboratory of Pathophysiology and Immunology of the D.F. Chebotarev Institute of Gerontology, NAMS of Ukraine.

To find the correlation between the NUC development and SNP of genes when analyzing the allele frequency, a multiplicative model was used in cases of observance of the Hardy-Weinberg equilibrium and a general inheritance model - when analyzing the frequency of genotypes when the equilibrium was not observed. Statistical analysis was performed using the EXCEL package of standard software for statistical analysis; to assess the reliability of differences in the groups, the Student's t criteria were used.

Results of the study and their discussion. Так как НЯК относится к полигенным заболеваниям, проанализировали частоту выявления SNP генов, ответственных за секрецию цитокинов и работу толлподобных рецепторов, которые могут способствовать активации воспаления, как наиболее важных в патогенезе НЯК. Для полноценного анализа возможного взаимодействия развития НЯК с вариантами SNP генов IL1 (T-31C), IL1 (T-511C), IL6 (C-174 G), IL10 (592C>A), IL10 (C-819T), IL10 (G-1082A), Tlr2 (Thr399ile), Tlr4 (Thr399ile), Tlr4 (Asp299Gly), проведен подсчет соответствия полученных данных равновесию Харди-Вайнберга (тест χ^2 при уровне значимости df=1).

Since NUC belongs to polygenic diseases, we analyzed the frequency of detecting SNP genes responsible for the secretion of cytokines and the work of toll-like receptors, which can promote the activation of inflammation, as the most important in the pathogenesis of NUC. For a complete analysis of the possible interaction of NUC development with SNP variants of the IL1 (T-31C), IL1 (T-511C), IL6 (C-174 G), IL10 (592C> A), IL10 (C-819T), IL10 (G -1082A), Tlr2 (Thr399ile), Tlr4 (Thr399ile), Tlr4 (Asp299Gly), the correspondence of the obtained data to the Hardy-Weinberg equilibrium was calculated (test χ^2 at a significance level of df = 1).

Among the examined patients with nonspecific ulcerative colitis, the Hardy-Weinberg condition was fulfilled both for the observed cases and for the control patients, except for substitutions (p <0.05). When assessing the compliance with this equilibrium in the frequency distribution of the alleles associations, for some SNP genes, no confirmation was found in the study and the control groups. The obtained results of the control group are presented in table 1.

**Nonobservance of the Hardy-Weinberg equilibrium in the control group
of healthy individuals**

No	gen	genotype	control	HWE	χ^2	p
1.	IL10 C-819T	Genotype C/C	0.449	0.525	7.09	0.008
		Genotype C/T	0.551	0.399		
		Genotype T/T	0.000	0.076		
2.	IL10 G-1082A	Genotype A/A	0.061	0.250	27.94	1.0E-7
		Genotype A/G	0.878	0.500		
		Genotype G G	0.061	0.250		
3.	Tlr4 Asp299Gly	Genotype A/A	0.245	0.387	18.03	2.0E-5
		Genotype A/G	0.755	0.470		
		Genotype G G	0.000	0.143		

As it is seen from the presented table, in the control group of healthy individuals, a shift in the genotypes frequency from the normal distribution was revealed: rs 1800871 (IL 10, $\chi^2 = 7.09$, $p < 0.008$); rs 100896 (IL 10, $\chi^2 = 27.94$, $1.0E-7$); rs 4986790 (Tlr4, $\chi^2 = 18.03$, $2.0E-5$), which indicates that there is no correspondence to the Hardy-Weinberg equilibrium for the SNPs of the IL10 (C-819T), IL10 (G-1082A), Tlr4 (Asp299Gly) genes.

Inconsistency with the Hardy-Weinberg equilibrium is also evident in the group of patients with NUC. According to the obtained results of the study, there was a shift in the frequency of genotypes from the normal distribution in the group of patients with IBD for SNP of IL10 (G-1082A) and Tlr4 (Asp299Gly) genes. Thus, in the group of patients with IBD, when analyzing the SNP data of the rs 1800896 (IL 10) gene, it was found that $\chi^2 = 5.18$, $p < 0.02$, and for SNP of the rs 4986790 (Tlr4) gene - $\chi^2 = 8.03$, $p < 0.005$, which confirms the impossibility of using the multiplicative model to identify the correlation between the development of NUC and SNP data, since the Hardy-Weinberg equilibrium is not observed. The correlation between the above genes SNP and NUC was analyzed using a general inheritance model taking into account the frequency of genotypes.

According to our data, the association of allele frequencies of genes with NUC was revealed by SNPs of genes IL1 (T-31C), IL1 (T-511C), IL10 (592C> A), Tlr4 (Thr399ile). The results are presented in table 2.

Table 2

Multiplicative inheritance model for a sample of patients with NUC

gen	allele	NUC	control	χ^2	p	value	OR95%CI
IL1rs1143627	C	0.538	0.673	3.92	0.05	0.56	0.32 – 1.00
	T	0.462	0.327				
IL1 rs 16944	C	0.491	0.357	3.71	0.05	1.73	0.99 – 3.04
	T	0.509	0.643				
IL10rs1800872	A	0.689	0.816	4.43	0.04	0.50	0.26 – 0.96
	C	0.311	0.184				
Tlr 4rs4986791	C	0.934	0.796	8.45	0.004	3.63	1.46 – 9.01
	T	0.066	0.204				

In accordance with the presented table, in patients with NUC, the correlation with the frequency of SNP alleles of genes responsible for the stability of the intestinal mucosa epithelium and regulation of cytokine production was revealed. The association of the disease was revealed by the multiplicative model of inheritance for substitutions of the T allele rs1143627 (IL1, $\chi^2 = 3.92$, $p < 0.05$), the C allele rs 16944 (IL1, $\chi^2 = 3.71$, $p < 0.05$), and the C allele rs1800872 (IL10, $\chi^2 = 4.43$, $p < 0.04$), allele C rs4986791 (Tlr 4, $\chi^2 = 8.45$, $p < 0.004$), compared to healthy persons.

The said genetic changes confirm the influence of the cytokine imbalance on the development and course of NUC, predict the possibility of a more severe and complicated course of the disease. The revealed correlation in patients with NUC with the SNP of the Tlr 4 gene (rs 4986791), C allele, confirms the influence of dysbiotic disorders on the development of the disease, possibly causing an inadequate response to the ongoing therapy.

When analyzing the general model of SNP substitutions inheritance in genes of patients with NUC, an associative relationship was revealed with 3 substitutions. The result is shown in table 3.

General inheritance model in patients with NUC

gen,SNP	genotype	genotype frequency		χ^2	p	OR	
		NUC	control			value	95%CI
Control group –healthy persons (n=49)							
IL10 rs1800896	A/A	0.302	0.061	10.21	0.006	6.63	1.79 –24.50
	A/G	0.623	0.878			0.23	0.08 – 0.64
	G/G	0.075	0.061			1.25	0.27 – 5.90
Tlr 4 rs4986791	C/C	0.868	0.633	8.30	0.02	3.82	1.43 –10.21
	C/T	0.132	0.327			0.31	0.12 – 0.85
	T/T	0.000	0.041			0.18	0.01 – 3.79
Tlr4 rs4986790	A/A	0.679	0.245	31.98	1.0E-7	6.53	2.74 –15.58
	A/G	0.208	0.755			0.08	0.03 – 0.22
	G/G	0.113	0.000			13.55	0.74 -247.18

According to the presented table, the highest associative risk of developing NUC was determined with carrying SNP in the Tlr4 gene (rs4986790). When compared to the control group, SNPs of the homozygous genotype G / G of the Tlr4 gene (rs4986790) prevailed in patients with NUC ($\chi^2 = 31.98$, $p < 0.05$, OR = 13.55, 95% CI: 0.74 - 247.18), which may indicate the correlation of this polymorphism with the risk of developing NUC, confirms the influence of microbiota stability on the course and development of pathological changes in the intestine, a possible tendency to dysbiotic disorders of the intestine.

In addition, in patients with NUC, the dominance of the wild homozygous genotype A / A in the IL10 gene (rs1800896) was revealed ($\chi^2 = 10.21$, $p < 0.006$, OR = 6.63, 95% CI: 1.79 - 24.50). Also, the obtained results of the analysis revealed an association of the risk of developing NUC with the SNP substitution carriership in the Tlr4 gene (rs4986791), wild type C / C genotype ($\chi^2 = 8.30$, $p < 0.02$, OR = 3.82, 95% CI: 1.43–10.21), and the data were consistent with the results of the multiplicative model of inheritance, which may confirm the special role of the above SNP in the pathogenesis of NUC development.

When using the multiplicative and general models of inheritance to analyze the possible predisposition to the development of NUC for the SNP variants of the IL6 (C-174 G) and Tlr2 (Thr399ile) genes, no association with the disease was found, compared to the control group of healthy individuals, which does not contradict the data of other authors [6, 7] and requires further diagnostic search. Identification of allelic polymorphisms of the genes responsible for the production of cytokines in patients with NUC can facilitate prescription of timely personalized adequate therapy, which will prevent further disease progression and the occurrence of complications in our patients. SNPs of genes in IBD can influence the development of an individual immune response and alter the production of cytokines responsible for the inflammatory process. In our patients, the correlation of the disease was revealed both with the frequency of the IL10 SNP of the wild genotype (rs1800896) and with the allelic polymorphism of T allele in the IL1 gene (rs1143627), C allele of the IL1 (rs 16944) and IL10 (rs1800872) genes, which confirms the data of foreign researchers [9, 12]. These changes can affect the work of the pleiotropic cytokines IL1 and IL10, play an important role in the process of inflammation and formation of the body's defensive reactions. In addition, the multifactorial effect of the genetic SNP in our patients was confirmed by the identification of the disease association with SNP of the Tlr4 gene (rs4986790), which regulates the mechanisms of pathogenic microorganisms recognition and, when they change, can also alter the immune response, contributing to an increase in the production of proinflammatory cytokines and chemokines. In contrast to foreign authors [7], in which associations of NUC with SNP of the Tlr4 gene (rs4986791) were only detected in allelic models, according to our data, the correlation was determined both by multiplicative and general inheritance models.

Most researchers of NUC, note the difficulties in diagnosing the disease. Timely identification of gene polymorphisms responsible for the stability of the intestinal mucosa epithelium, such as SNP of the Tlr4 gene, secretion of pro-inflammatory and anti-inflammatory cytokines (IL1, IL10), will permit earlier suspecting the patient's tendency to IBD, the possibility of this disease onset, and timely prescribing appropriate adequate therapy.

Conclusion

Thus, the results obtained confirm the relationship between the development of NUC and SNP of genes responsible for the production of cytokines and the epithelium stability of the colon mucosa. In the examined patients, an association was found between the development of NUC with the frequency of wild genotype SNPs in the IL10 gene (rs1800896), homozygous G / G genotype SNPs of the Tlr4 gene (rs4986790), the frequency of the T allele in the IL1 gene (rs1143627), C allele in the IL1 (rs 169) and

IL10 (rs1800872) genes, which contributed to the disturbance and imbalance in the production of cytokines, predisposing to the development of NUC and aggravating the disease course. Associations for the SNP of the Tlr4 gene (rs4986791) with the development of NUC were revealed both by the multiplicative model with the C allele and by the general inheritance model with the wild type C / C genotype, which confirms the importance of this SNP in the development of the disease.

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Реферати

РОЛЬ ОДНОНУКЛЕОТИДНЫХ ПОЛИМОРФИЗМОВ ГЕНОВ У РАЗВИТИИ НЕСПЕЦИФИЧЕСКОГО ВЯЗВОВОГО КОЛИТУ

Кир'ян О.А., Дорофєєв А.Е., Хайменова Г.С.,
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Метою дослідження було вивчення особливості впливу поліморфних варіантів генів IL1 (T-31C), IL1 (T-511C), IL6 (C-174 G), IL10 (592C> A), IL10 (C-819T), IL10 (G-1082A) Tlr2 (Thr399ile), Tlr4 (Thr399ile), Tlr4 (Asp299Gly) на розвиток неспецифічного виразкового коліту у хворих. В обстеження було залучено 53 пацієнта, контрольну групу складала випадкова вибірка з 49 здорових осіб. У наших пацієнтів виявлено асоціацію розвитку неспецифічного виразкового коліту з частотою поліморфного варіанту гена IL10 дикого генотипу (rs1800896), гомозиготного генотипу G/G поліморфного варіанту гена Tlr4 (rs4986790), частотою алелі Т в гені IL1 (rs1143627), алелі С в генах IL1 (rs 16944) і IL10 (rs1800872), які сприяли порушенню і дисбалансу у виробленні плейотропних цитокінів IL1 і IL10, модифікуючи перебіг неспецифічного виразкового коліту, впливаючи на його розвиток. В роботі показані асоціативні зв'язки для однонуклеотидного поліморфізму гена Tlr4 (rs4986791) з розвитком неспецифічного виразкового коліту як по мультипликативній моделі з алеллю С, так і за загальною моделлю успадкування з диким типом генотипу C/C, що підтверджує важливість даного однонуклеотидного поліморфізму в розвитку захворювання.

Ключові слова: неспецифічний виразковий коліт, однонуклеотидний поліморфізм генів, аллель, генотип.

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РОЛЬ ОДНОНУКЛЕОТИДНЫХ ПОЛИМОРФИЗМОВ ГЕНОВ В РАЗВИТИИ НЕСПЕЦИФИЧЕСКОГО ЯЗВЕННОГО КОЛИТА

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Целью нашего исследования было изучить особенности влияния полиморфных вариантов генов IL1 (T-31C), IL1 (T-511C), IL6 (C-174 G), IL10 (592C>A), IL10 (C-819T), IL10 (G-1082A), Tlr2 (Thr399ile), Tlr4 (Thr399ile), Tlr4 (Asp299Gly) на развитие неспецифического язвенного колита у больных. Были обследованы 53 пациента с неспецифическим язвенным колитом, контрольную группу составила случайная выборка из 49 здоровых людей. Выявлена ассоциация развития неспецифического язвенного колита с частотой однонуклеотидного полиморфизма гена IL10 дикого генотипа (rs1800896), гомозиготным генотипом G/G однонуклеотидного полиморфизма гена Tlr4 (rs4986790), частотой аллеля Т в гене IL1 (rs1143627), аллеля С в генах IL1 (rs 16944) и IL10 (rs1800872), которые способствовали нарушению и дисбалансу в выработке плейотропных цитокинов IL1 и IL10, предрасполагая к развитию неспецифического язвенного колита и усугубляя течение болезни. В работе показаны ассоциативные связи для однонуклеотидного полиморфизма гена Tlr4 (rs4986791) с развитием неспецифического язвенного колита как по мультипликативной модели с аллелью С, так и по общей модели наследования с диким типом генотипа C/C, что подтверждает важность данного однонуклеотидного полиморфизма в развитии заболевания.

Ключевые слова: неспецифический язвенный колит, однонуклеотидный полиморфизм генов, аллель, генотип.

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SUBSTANTIATION OF A SAFE PERIOD OF TIME FOR THE ORAL CAVITY SANITATION UNDER GENERAL ANESTHESIA IN CHILDREN AGED 12-18 YEARS

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The article substantiates a safe period of time for the sanitation of the oral cavity in an outpatient setting under general anesthesia in children aged 12-18 years in order to prevent the occurrence of cognitive dysfunction of the brain against the background of hypoxic lesions. The results of the study revealed: in order to prevent the occurrence of brain hypoxia during dental sanitation of the oral cavity in conditions of general anesthesia (without intubation), children aged 12 to 18 years should spend within 60±15 minutes; the contraindication for the planned sanitation of the oral cavity in conditions of general anesthesia (without intubation) is the absence of a history of acute respiratory diseases for at least 2 weeks; if there is a history of acute respiratory disease in less than 2 weeks and an acute dental need, ambulance in general anesthetic conditions (without intubation) is possible within ≤20 min.

Key words: cerebral oximetry, SpO₂, rSO₂, general anesthesia.

The work is a fragment of the research projects: "Diagnosis, treatment and prevention of diseases of the hard tissues of the teeth at different stages of their development in children", state registration No. 0116U000122, and "Features of the clinic, diagnosis, treatment and prevention of dental diseases in children with developmental disabilities", state registration No. 0119U100454.

The high intensity and prevalence of dental caries in the pediatric population, create unfavorable conditions for maintaining the psychological balance of the child at the dental attendance, and hence its further mental development. Most children during the dentist's attendance are accompanied by negative stress, which can lead to impaired cognitive function of the brain [7, 8].

According to various authors, about 70% of school-age children and about 90.9% of preschool children require psychological correction before dental intervention [6].

The result of the absence of psychological contact between the dentist and a small patient is the unreasonable expansion of indications for the application of general anesthesia in the oral cavity sanitation in children of all ages [3].

In order to preserve the child's psychological sphere, dentists sometimes underestimate the risks of general anesthesia. Today, a large number of scientific papers are devoted to the study of the general anesthesia negative impact on cognitive function in patients of all ages [1, 9, 10, 11]. One of the first places in the statistics of anesthesiologic complications is occupied by cognitive disorders in hypoxic brain lesions [1, 4, 13]. Therefore, in order to prevent the occurrence of cognitive impairment of the brain against the background of its hypoxic lesions during the oral cavity sanitation in children under conditions of general anesthesia, along with providing adequate indicators of vital functions of the organism, such as heart rate, blood pressure, pulse oximetry, oxygen status of the brain. A large number of methods for the evaluation of cerebral hemodynamics have been described in the literature [4, 5]. However, there is no data on the study of brain oxygen status in children during outpatient interventions under general anesthesia, which may be important to prevent cognitive impairment in children against hypoxic brain damage in dental intervention.

The purpose of the study was to establish a safe period of time for the oral cavity sanitation in outpatient conditions under general anesthesia in children aged 12-18 years in order to prevent the occurrence of cognitive dysfunction of the brain against the background of hypoxic lesions.

Materials and methods. Sanitation of the oral cavity in the conditions of general anesthesia was carried out in 30 children aged from 12 to 18 years on the basis of the Dental Medical Center at the Bogomolets National Medical University. According to the classification of temperaments (Thomas and Chess, 1997), this group of children included: 19 children with "difficult" temperament and 11 children – with "slow-to-warm-up" temperament.

Assessment of the oxygen status of the brain during the oral cavity sanitation under the conditions of general anesthesia was performed using a non-invasive method of neuromonitoring – cerebral oximetry (apparatus for arterial blood gas monitoring: 4-channel regional oximeter with EQUANOXTM technology, Bluetooth wireless technology and 232 wireless technology (Model 7600) (State Registration Certificate No. 12580/2013. Manufacturer: Nonin Medical, Inc., USA).

To predict cognitive changes against the background of hypoxic brain disorders, we used the results of J. Meixensberger 1998 [2].

Results of the study and their discussion. Dynamics of vital functions indices monitoring of an organism in children of different temperaments at the age from 12 to 18 years are presented in table. 1.

Table 1

Dynamics of vital functions indices monitoring of an organism in children aged 12-18 years

Stages of operation		Before the induction	Induction	Treatment	After treatment	Overall indices	
Temperament	Monitoring indicators						
“Difficult” (n=19)	rSO ₂ (%)	68.26±1.56	73.31±1.89	75.17±2.21	75.46±1.56	73.05±1.03	
	SpO ₂ (%)	96.11±1.05	97.32±1.29	96.62±2.15	95.37±1.3	96.35±1.07	
	BP (mmHg)	SBP	124.3±2.58	114.2±5.5	112.1±5.01	108.2±1.65	114.69±1.84
		DBP	81.11±1.59	75.05±3.37	72.26±3.63	72.21±3.26	75.16±2.47
	HR (bpm)	110.9±5.59	105.6±4.08	98.11±1.24	96.84±1.21	102.9±2.39	
“Slow-to-warm-up” (n=11)	rSO ₂ (%)	70.18±2.52	76.34±0.85	76.48±1.62	77.49±0.55	75.12±1.03	
	SpO ₂ (%)	95.62±0.8	96.18±1.33	96.65±1.19	96.64±0.5	96.27±0.74	
	BP (mmHg)	SBP	112.2±1.99	110.5±1.92	111.8±2.09	108.9±0.94	113.1±1.11
		DBP	80.09±1.81	72.45±1.97	71.64±3.04	72±3.66	74.05±2.05
	HR (bpm)	108.4±1.21	101.6±3.07	98.36±1.74	96.73±1.01	101.27±1.4	
Overall indices (n=30)	rSO ₂ (%)	68.97±2.14	74.42±2.16	75.65±2.09	76.21±1.39	73.81±1.44	
	SpO ₂ (%)	95.93±0.98	96.9±1.39	96.63±1.83	95.83±1.23	96.32±0.95	
	BP (mmHg)	SBP	123.1±2.79	112.8±4.48	112±4.13	108.5±1.45	114.11±1.77
		DBP	80.73±1.72	74.1±3.17	72.03±3.39	72.13±3.35	74.75±2.35
	HR (bpm)	110±4.64	104.1±4.17	98.2±1.42	96.8±1.13	102.28±2.16	

As it can be seen from the table. 1, there was a decrease in HR and BP in the preoperative period. These figures were 110±4.64 b.p.m. and 123.1±2.79/80.73±1.72 mm.Hg, respectively.

The values of cerebral oximetry were significantly observed in the preoperative period (rSO₂=68.97%±2.14), compared with the operative periods (rSO₂=75.65±2.09) in the presence of equally exact pulse oximetry indices. SpO₂ indices left 95.93%±0.98 and 96.63%±1.83 respectively.

Thus, it can be argued that general anesthesia in children with dental intervention was a kind of psychological “protection” of higher nervous activity from the stress effects.

The results of cerebral oximetry during dental intervention in conditions of general anesthesia in children aged 12-18 years are presented in fig. 1.

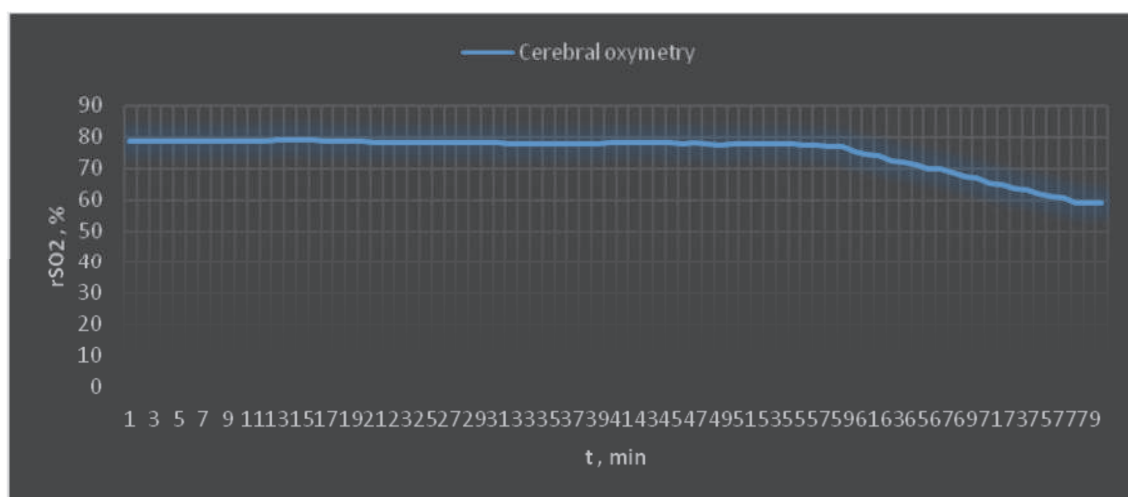


Fig. 1. Indices of rSO₂ in children aged 12-18 years in the period of oral cavity sanitation under general anesthesia (without intubation).

The decrease in rSO₂ started from the 60th minute. The minimum rSO₂ value was 60.59%±0.58, which is within the normal range, and was 77 minutes (table 2).

The maximum value of rSO₂ was 79.11%±2.02, accounting to 13; 15 min. and did not exceed the permissible maximum value (90.78%) (table 2).

Thus, the progressive decrease in cerebral oximetry indicated that in order to avoid complications after treatment, children aged 12 to 18 years could be rehabilitated by oral anesthesia (without intubation) within 60±15 min.

Min and max rSO2 indices in children aged 12-18 years

Mean value n =30	“Difficult” n=19	“Slow-to-warm-up” n=11
max=90.78%	max=90.2%	max=91.74%
75.65% (20%=15.13%)	75.17% (20%=15.03%)	76.48% (20%=15.26%)
min=60.52%	min=60.14%	min=61.22%

Respiratory complications were observed in 3.33% of children during dental invasion. The results of rSO2 and their correlation with SpO2 are presented in fig. 2, 4.

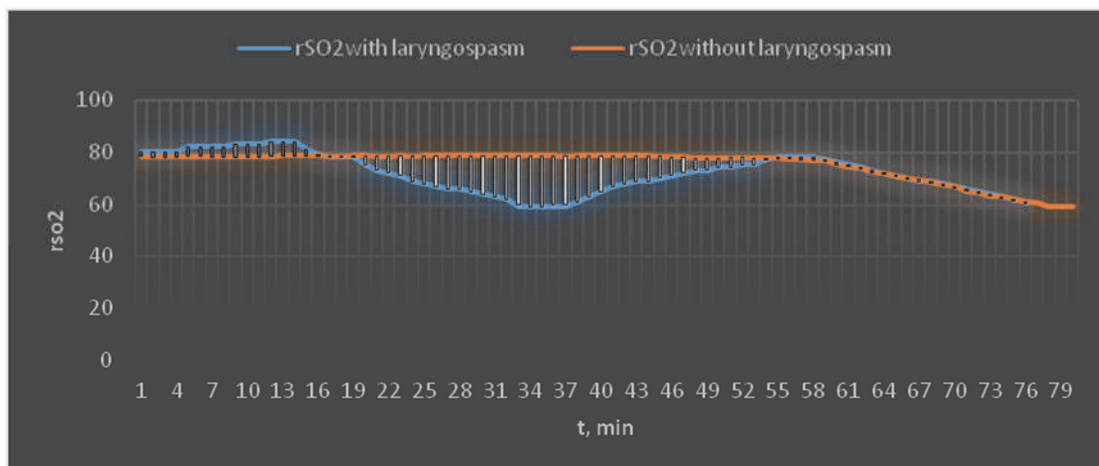


Fig. 2. Dynamics of rSO2 in laryngospasms during the oral cavity sanitation under general anesthesia (without intubation) in children aged 12-18 years.

The mean rSO2 value in children with laryngospasm during oral cavity sanitation under general anesthetic conditions was $72\% \pm 7.27$ – decreased by 4.82% relative to the mean rSO2 of the relevant age group ($rSO2 = 75.65\% \pm 2.09$). Decreasing rSO2 from 20-53min. (<20 min) was 6.12% ($rSO2 = 67.59\% \pm 5.16$). The maximum decrease was observed at 34-35min. ($rSO2 = 59\% \pm 0.01$), which is 18.05% of the total value and 12.71% of the rSO2 index directly in laryngospasm.

Among children with complications in the form of laryngospasm during the oral cavity sanitation under the conditions of general anesthesia had a history of 100% less than 2 weeks after complete recovery of the acute respiratory disease (ARD). To study the effect of respiratory tract inflammation on the possibility of complications, we compared the group of children with a history of ARD ≤ 2 weeks with the group of children who had a history of ARD for ≥ 2 weeks but ≤ 4 weeks (16.67%).

The results of this analysis are presented in fig. 3.

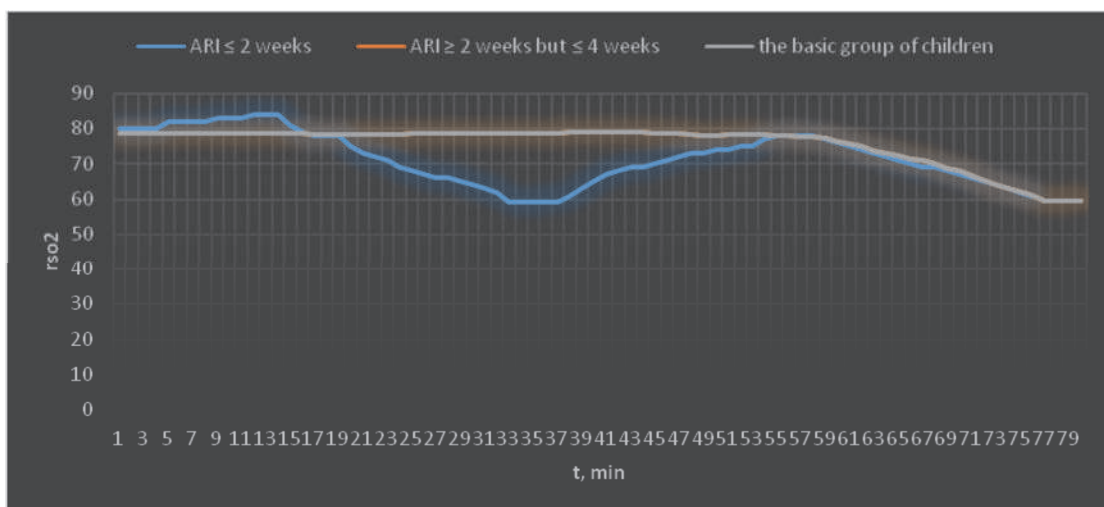


Fig. 3. Indices of rSO2 in children 12-18 years of age with ARI ≤ 2 weeks (I) and ARI of ≥ 2 weeks but ≤ 4 weeks (II) and their correlation with baseline groups of children (III).

We noted the decrease in rSO2 ($72\% \pm 7.27$) by 4.28% in Group I, while the rSO2 in Groups II and III were not significantly different and were $75.78\% \pm 5.66$ and $75.65\% \pm 2.09$ respectively.

In the study of indices of cerebral oximetry and pulse oximetry, no correlation of these indices in time and magnitude was found. The mean value of SpO₂=95.12%±3.34 with the mean value for this age group being 96.63%±1.83 (fig. 4).

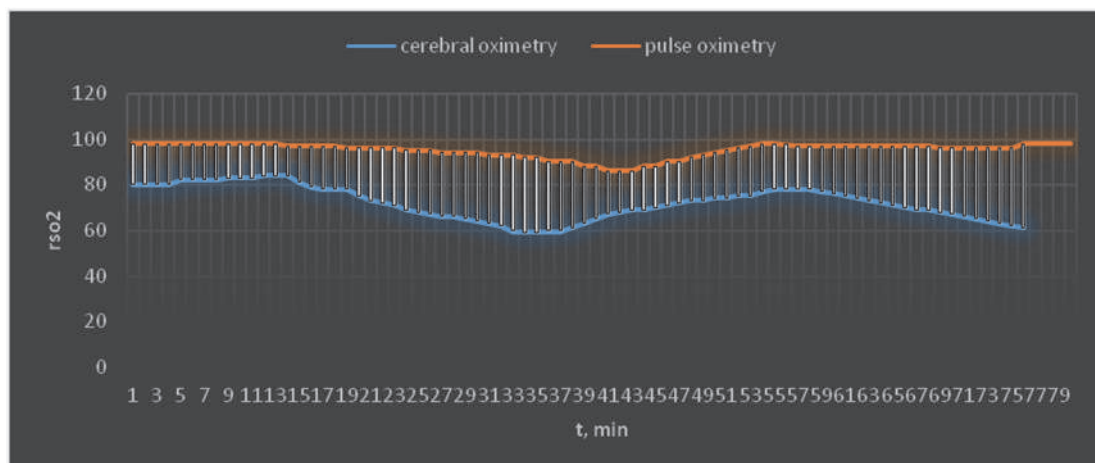


Fig. 4. Correlation of rSO₂ and SpO₂ in complications during oral remediation under general anesthesia (without intubation) in children aged 12-18 years.

Reduction of SpO₂ (88.33%±1.67) in complications occurred from 35 min. to 47 minutes (<20 min.) by 8.59%, which is 15 minutes later respect to rSO₂ (20-53 min.) and almost twice the magnitude (rSO₂ decreased by 4.82%).

In general, our results are consistent with the data of other researchers [1, 4, 5]. However, in our aspect, the problem of oral sanitation in children under general anesthesia has been studied only partially [9]. We tried to find the optimal period of time for general anesthesia of children aged 12-18 years under in an outpatient setting to prevent the occurrence of cognitive dysfunction in the background of cerebral hypoxia. However, there is data with which our study is partially consistent [4].

Conclusions

1. In order to prevent the occurrence of brain hypoxia during dental sanitation of the oral cavity under the conditions of general anesthesia (without intubation), children aged 12 to 18 years should spend within 60±15 minutes.
2. The contraindication for the planned sanitation of the oral cavity in conditions of general anesthesia (without intubation) is the absence of a history of acute respiratory diseases for at least 2 weeks.
3. If there is a history of acute respiratory disease in less than 2 weeks and an acute dental need, ambulance in general anesthetic conditions (without intubation) is possible within ≤20 min.
4. SpO₂ indices do not correlate with rSO₂ values.
5. The method of cerebral oximetry is more sensual than the method of pulse oximetry, which makes it possible to detect changes in oxygen balance of the cerebral cortex in a timely manner and to maintain it in time.

Prospects for further research lie in the fact that to compare the dynamics of cerebral oximetry in children of different ages during dental interventions under general anesthesia in an outpatient setting and during various dental outpatient manipulations. According to the results of the study, establish a safe period of time for different dental manipulations for different types of dental sanitation in children of different ages, in order to determine the indications for dental intervention in conditions of general anesthesia on an outpatient basis.

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Реферати

ОБГРУНТУВАННЯ БЕЗПЕЧНОГО ПРОМІЖКУ ЧАСУ ПРОВЕДЕННЯ САНАЦІЇ ПОРОЖНИНИ РОТА ПІД ЗАГАЛЬНИМ ЗНЕБОЛЕННЯМ У ДІТЕЙ ВІКОМ 12-18 РОКІВ

Коваль О. І.

В статті обгрунтовано безпечний проміжок часу проведення санації порожнини рота в амбулаторних умовах під загальним знеболенням у дітей віком 12-18 років з метою попередження виникнення когнітивних дисфункцій головного мозку на фоні гіпоксичних уражень. За результатами дослідження встановлено: з метою попередження виникнення гіпоксії головного мозку при проведенні стоматологічної санації порожнини рота в умовах загального знеболення (без інтубації) дітям віком від 12-ти до 18-ти років варто проводити в межах 60 ± 15 хв.; протипоказанням для планового проведення санації порожнини рота в умовах загального знеболення (без інтубації) є відсутність в анамнезі гострих респіраторних захворювань не менш ніж за 2 тижні; при наявності в анамнезі гострих респіраторних захворювань менш ніж за 2 тижні та гострою стоматологічною потребою, надання швидкої допомоги в умовах загального знеболення (без інтубації) можливо в межах ≤ 20 хв.

Ключові слова: церебральна оксиметрія, SpO₂, rSO₂, загальне знеболення.

Стаття надійшла 26.08.2019 р.

ОБОСНОВАНИЕ БЕЗОПАСНОГО ПРОМЕЖУТКА ВРЕМЕНИ ПРОВЕДЕНИЯ САНАЦИИ ПОЛОСТИ РТА ПОД ОБЩИМ ОБЕЗБОЛИВАНИЕМ У ДЕТЕЙ В ВОЗРАСТЕ 12-18 ЛЕТ

Коваль О. И.

В статье обоснован безопасный промежуток времени при проведении санации полости рта в амбулаторных условиях под общим обезболиванием у детей в возрасте 12-18 лет с целью предупреждения возникновения когнитивных дисфункций головного мозга на фоне гипоксических поражений. По результатам обследования установлено: с целью предупреждения возникновения гипоксии головного мозга стоматологическую санацию полости рта в условиях общего обезболивания (без интубации) детям в возрасте от 12-ти до 18-ти лет следует проводить в пределах 60 ± 15 мин. Противопоказанием для планового проведения санации полости рта в условиях общего обезболивания (без интубации) является отсутствие в анамнезе острых респираторных заболеваний не менее чем за 2 недели. При наличии в анамнезе острых респираторных заболеваний менее чем за 2 недели и острой стоматологической необходимости оказание скорой помощи в условиях общего обезболивания (без интубации) возможно в пределах ≤ 20 мин.

Ключевые слова: церебральная оксиметрия, SpO₂, rSO₂, общее обезболивание.

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APPLICATION OF NANOCRYSTALS IN TREATMENT OF CHRONIC APICAL PERIODONTITIS

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The use of nanocrystals for the treatment of chronic apical periodontitis is the current level of nanomedicine. We examined and treated 11 patients aged 17 to 67 years for aggravated and chronic apical periodontitis using the author's method of using phosphate buffer. An assessment of clinical and radiological data was carried out, which confirmed the process of restoration of periapical tissues in 11 patients. She showed that the regeneration of the apical and adjacent areas of the periodontal in 10 cases had a positive trend already after 29-30 days from the start of treatment; in 1 case, there was a slowed down dynamics of recovery after 40 days from the start of treatment due to the presence of somatic pathology in the patient. The proposed method for the treatment of chronic apical periodontitis on the basis of the obtained treatment results requires further study in the long term with the aim of widespread implementation in practical dentistry.

Key words: treatment of chronic apical periodontitis, single crystals, phosphate buffer.

The work is a fragment of the research project "Application of modern technologies in diagnostics and treatment for rehabilitation of dental patients by orthopedic methods", state registration No. 0117U004778.

Nanomedicine, a novel branch of medicine, applies nanotechnological advances for the treatment of various diseases in dental patients with control of biological activity of the outcomes. Currently, for non-cellular strategy of tissue engineering in the treatment of apical periodontitis, application of nanocarriers is crucial [7, 12]. In endodontics, the diverse solutions, including 10%, 15%, 40% citric acid, are used at the stage of preparation of root canals by mechanical, instrumental and chemical removal of organic debris,

necrotic masses and dentin residues. Emphasizing one of the characteristic properties of citric acid in relation to its interaction with the hard tooth tissue, the study has established that the above reaction is the side-formation of $\text{Ca}_3(\text{C}_3\text{H}_5\text{O}_7)_2$ calcium citrate nanocrystals [5].

A similar process occurs during the filling of teeth with composite materials with preliminary phosphoric acid etching of enamel and dentin. Normally, phosphoric acid contributes to the formation of calcium phosphate, which is poorly soluble in water, or from a soluble state may become insoluble, i.e., the process depends on the state of chemical equilibrium [2, 8, 9, 11]. In this way, the donor of calcium ions is the own minerals of the hard tooth tissues [6]. Importantly, natural crystals of dentin and enamel contain calcium phosphate. As a result of chemical and physiological processes in the crown and root portions of the tooth, which are permeated with inter-prismatic spaces of the enamel and dentin tubules, a constant filling-up and circulation of calcium-rich cerebrospinal fluid occurs [3]. The latter, entering into reaction with phosphates, can form a characteristic biologically inert complex substance of calcium phosphate from the apex of the root to its crown [3, 12].

The density of the formed substance depends on a certain concentration of phosphate solution in the form of a buffer as a constant component of the pH level [6]. In the human body it is difficult to achieve the desired concentration of the above solution, since, as a result of physiological factors cerebrospinal fluid is constantly circulating in the root canal of the tooth leading to a certain inactivation of this solution [1, 4, 10]. Therefore, to reach such stability is possible only with the use of phosphate buffer. The method of preparation of the latter and the method of treatment are described in the declaration patent of Ukraine [6].

The search for novel non-toxic impregnation method continues to date, which makes the study relevant.

The purpose of the paper was at enhancement of the method of treatment of chronic apical periodontitis by obturation of the apex of the root using a white clay-based phosphate-buffered paste.

Materials and methods. 11 patients aged 17 to 67 years have been examined and received appropriate treatment for acute and chronic apical periodontitis according to the proposed technique. All patients were examined according to the traditional scheme; informed consents were obtained to be included in the experimental group. Additional examination involved radiological diagnostics before and after treatment, the duration of which was determined individually and dependant on the dynamics of individual treatment of the relevant clinical case within the time period of several days to several weeks. X-ray evaluation in both cases was performed by visual inspection under a magnifying glass of spot images, highlighting the dynamic changes of the periapical focus of destruction, which enlarged with exacerbation or diminished during the stabilization of the process.

The treatment technique included application of the proposed phosphate buffer, which was prepared (ex tempore) as follows: first, in a dental glass crucible, 13 drops of 15% solution of ammonium phosphate ($(\text{NH}_4)_3\text{PO}_4$) were mixed with 1 drop of phosphoric acid solution (37%, H_3PO_4), which is included in the set of any kit of materials for hard tooth tissues etching. The mixture, in the required amount, was transferred from the crucible to the dental glass using a plastic spatula and mixed with white clay (quantum satis) to form a paste of creamy consistency. Subsequently, properly prepared tooth should be isolated to avoid moisture of saliva and dried. Root canals, which, during the preparation of the tooth, could not be processed instrumentally, should be treated to the maximum possible length and expand their orifices cone-shaped. The obtained paste was injected to the enlarged orifices and into the root canal in a forcing manner using the root needle or canal filler. A temporary filling was made. The next visit was appointed following 24 hours or other time (at the discretion of a dentist). On the second visit, the temporary filling was removed. The paste was carefully removed from the cavity and root canal, washed with water and dried. Root canal filling can be performed with endodontic material according to the conventional technique or at the dentist's discretion and insulation gasket is placed. In periodontitis, the carious cavity was formed according to the requirements, and treatment was completed by filling the root canal and then the tooth cavity.

Results of the study and their discussion. All patients experienced reduction of the characteristic symptoms in the form of causal toothache when eating and unpleasant sensations of various kinds. In some cases, it was radiologically established that noticeable fibrotization and induration of destructive areas of the apical periodontium occurred at different times, starting from 2-3 days to several weeks.

In the approximate period of treatment, dental X-rays showed some clarification of the previously shadowed apical areas of the periodontium, and compaction of their contours around the perimeter of the focus of bone tissue destruction. In this case, along the perimeter of the pathological process, more distinct contours of differentiation of healthy and affected tissue were apparent.

For illustration, we report a case of treatment of the 65 year-old female patient K., who, at the first visit, was diagnosed with exacerbation of chronic apical periodontitis of tooth 45. Dental X-ray, presented in fig. 1a, shows a pronounced zone of bone tissue destruction in the form of a characteristic shadowing with blurred edges around the perimeter of the site of inflammation (marked with a line) in the area of the apex of the root. At the next visit, the patient almost had no complaints. Clinical examination showed a significant reduction in symptoms compared to the first visit.

Following 2 days after treatment the dental X-ray, presented in fig. 1b, showed compaction of the focus of destruction in the form of characteristic clarification in the form of certain islets of irregular shape and different shades. This heterogeneity of shades characterized the mineralization zones of cancellous bone tissue, localized in this area of the alveolar process. A detailed examination of the area at root apex revealed clarification of irregular form, which diffusely interspersed with adjacent tissues (marked with an arrow). We hypothesize that this is the vascular-nervous bundle of the central root canal. The latter is directly involved in the trophism of the tooth canal and periodontium by circulating calcium-rich dentinal cerebrospinal fluid. It is confirmed by the reaction of the latter with phosphate buffer, which leads to the corresponding mineralization of calcium phosphate formed in the specified area.

Certain features of positive dynamics (fig. 2) were observed in the treatment of chronic granulomatous periodontitis. For illustration, we report a clinical case of treatment of the 66 year-old male patient K., who, upon appropriate examination, was diagnosed with chronic granulomatous periodontitis of tooth 35. The dental X-ray, presented in fig. 2a, showed the oblong granuloma with shadowed edges (marked with an arrow) at the apex of the tooth root.

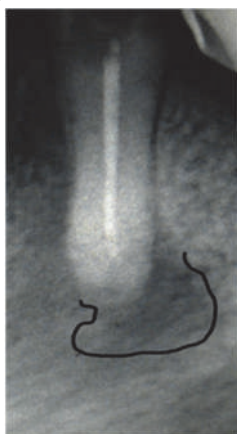


Fig. 1. Spot dental X-ray of tooth 45 of the 65 year-old female patient K. The state of the tooth before treatment (a). The state of the tooth following 2 days after treatment (b).

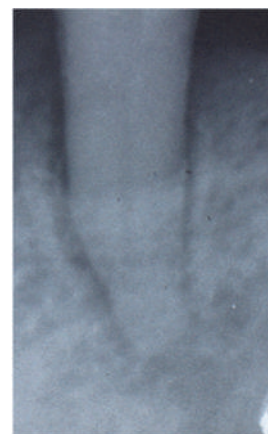
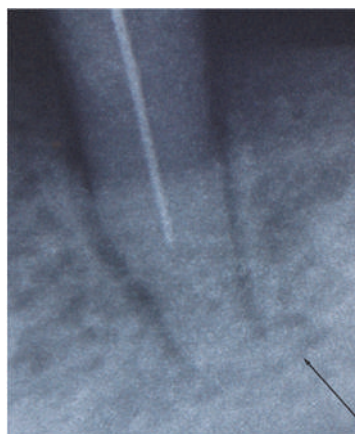


Fig. 2. Spot dental X-ray of tooth 35 of the 66 year-old male patient K. The state of the tooth before treatment (a). The state of the tooth following 4 days after treatment (b).

Periodontal fissure on both sides of the root is enlarged and has shadowed contours in the form of a dotted strip. The dental X-ray, presented in fig. 2b, showed that granuloma lost its contours following 4 days after treatment and an induration in the form of a characteristic clarification of the tissues at the apex of the root and periodontal fissure was noted. The latter almost lost its dottedness and was mineralized on both sides of the tooth root.



Fig. 3. The 52 year-old male patient T. Image of the maxillary central incisors at the stage of clinical examination (3a). Spot dental X-ray of both maxillary central incisors at the stage of clinical examination (3b).

The clinical case (fig. 3) of the intermediate stage of treatment of tooth 21 of the 52 year-old male patient T., who was diagnosed with chronic granulomatous periodontitis 21, Class I tooth mobility, is of special attention.

Tooth 21 has not been treated for more than 30 years after acute mechanical injury. Current X-ray examination of another central incisor revealed crack in the root, though the tooth has physiological mobility.

A detailed study of the spot dental X-ray of tooth 21 (fig. 4a) revealed a significant area of bone destruction in the area of the root apex within 3.9 mm (marked with horizontal arrows). The structure of the dentin of the latter has largely lost its density in the form of a characteristic shadowing without clear

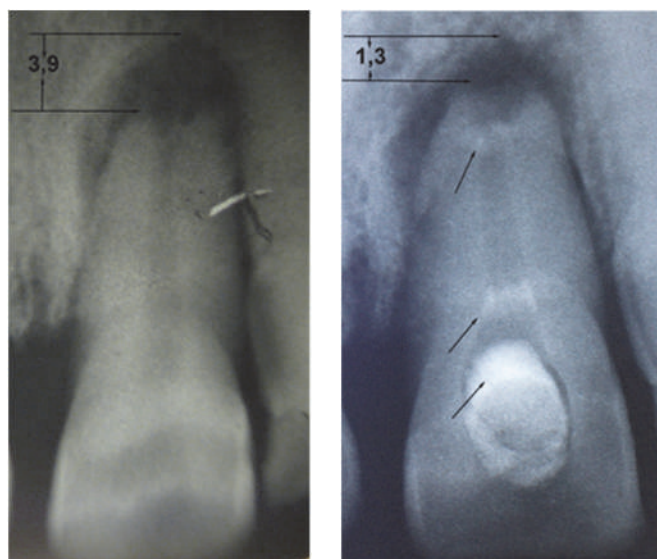


Fig. 4. Spot dental X-ray of tooth 21 of the 52 year-old male patient T. The state of the tooth before treatment (a). The state of the tooth following 15 days after treatment (b).

boundaries. The periodontal fissure is enlarged on both sides of the root in the form of a clearly defined dark stripe, which from the distal side of the root reaches the neck of the tooth.

The dental X-ray of the tooth following 15 days after treatment is presented on Fig. 4b. The study revealed diminishing of the shadowed area at the apex of the root to 1.3 mm (marked with horizontal arrows) and a clearer dissociation from the bone tissue, which indicates a positive process of mineralization of the latter. The active zones of mineralization were clearly distinguished in the thickness of the root dentin in the form of clear X-ray contrast bands of different widths at the apex and orifice of the canal (marked with diagonal arrows). The phosphate-buffered paste contrasted especially

clearly in the tooth cavity. The different degree of contrast of the above active zones of mineralization, in our opinion, indicated a different degree of presence of dentinal cerebrospinal fluid in the tubules, which in their histological structure are more open towards the central canal of these zones. During the treatment period the anatomical apex of the root also acquired a characteristic clear shape, and its surface had light and clear boundaries. The periodontal fissure became smaller and lighter due to mineralization. However, clinically, the mobility of the tooth has not changed.

Conclusion

The analysis of the clinical and radiological data of the regeneration process of periapical tissue during treatment of patients with chronic apical periodontitis has shown positive dynamics of regeneration of the apical and adjacent areas of the periodontium in all cases. The effectiveness of the developed treatment technique of above patients was confirmed by the results of clinical examinations and radiological methods of study. Thus, the proposed method of treatment of chronic apical periodontitis on the basis of approximate results of treatment requires further study in the long term to be widely implemented in practical dentistry.

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Реферати

**ЗАСТОСУВАННЯ НАНОКРИСТАЛІВ
ДЛЯ ЛІКУВАННЯ ХРОНІЧНОГО
ВЕРХІВКОВОГО ПЕРІОДОНТИТУ**Костиренко О.П., Мельник В.Л., Шевченко В.К.,
Силенко Ю.І., Єрошенко Г.А.

Застосування нанокристалів для лікування хронічного верхівкового періодонтиту – сучасний рівень наномедицини. Нами обстежено та проліковано 11 хворих віком від 17 до 67 років на загострений та хронічний верхівковий періодонтит за допомогою авторської методики з використанням фосфатного буферу. Проведена оцінка клінічних та рентгенологічних даних підтвердила процес відновлення периапікальних тканин у 11 пацієнтів. Вона показала, що регенерація верхівкових та прилеглих до них ділянок періодонту в 10 випадках мали позитивну динаміку вже через 29-30 днів від початку лікування; у 1 випадку – відмічена сповільнена динаміка відновлення через 40 днів від початку лікування через наявність у пацієнта соматичної патології. Запропонований спосіб лікування хронічного верхівкового періодонтиту на підставі отриманих результатів лікування потребує подальшого вивчення у віддалені терміни з метою широкого впровадження в практичну стоматологію.

Ключові слова: лікування хронічного верхівкового періодонтиту, монокристали, фосфатний буфер.

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**ПРИМЕНЕНИЕ НАНОКРИСТАЛЛОВ
ДЛЯ ЛЕЧЕНИЯ ХРОНИЧЕСКОГО
ВЕРХУШЕЧНОГО ПЕРИОДОНТИТА**Костыренко А.П., Мельник В.Л., Шевченко В.К.,
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Применение нанокристаллов для лечения хронического верхушечного периодонтита - современный уровень наномедицины. Нами обследовано и пролечено 11 больных в возрасте от 17 до 67 лет на обострившийся и хронический верхушечный периодонтит с помощью авторской методики использования фосфатного буфера. Проведена оценка клинических и рентгенологических данных, которые подтвердили процесс восстановления периапикальных тканей у 11 пациентов. Она показала, что регенерация верхушечных и прилегающих к ним участков периодонта в 10 случаях имели положительную динамику уже через 29-30 дней от начала лечения; в 1 случае - отмечена замедленная динамика восстановления через 40 дней от начала лечения из-за наличия у пациента соматической патологии. Предложенный способ лечения хронического верхушечного периодонтита на основании полученных результатов лечения требует дальнейшего изучения в отдаленные сроки с целью широкого внедрения в практическую стоматологию.

Ключевые слова: лечение хронического верхушечного периодонтита, монокристаллы, фосфатный буфер.

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O.O. Bogomolets National Medical University, Kyiv**DIETARY CORRECTION OF HYPERINSULINEMIA AND HEMOSTASIS PARAMETERS
IN OVERWEIGHT ARTERIAL HYPERTENSION PATIENTS**

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The purpose of our study was to investigate the influence of a carbohydrate-restricted diet up to 250 g per day for 12 weeks on body mass index, insulin level, and parameters of hemostasis in patients with arterial hypertension and metabolic disorders. It was registered the decrease in the body mass index by 7%, insulin level by 27.8%, factor von Willebrand activity ($p=0.009$) and soluble fibrin content ($p=0.03$) and the improvement of the platelet aggregation induced by ADP and ristocetin in patients with arterial hypertension and increased body mass index who followed the diet with carbohydrate restriction up to 250 g per day for 12 weeks in addition to standard therapy. A diet with carbohydrate restriction up to 250 g per day should be recommended to the patients with combination of arterial hypertension, increased body mass index and hyperinsulinemia in addition to antihypertensive therapy.

Key words: arterial hypertension, carbohydrate-restricted diet, body mass index, insulin level, hemostasis parameters.

The study is a fragment of the research project "Hemodynamic and coronary blood supply disturbances and ectopic myocardial activity in patients with ischemic heart disease and diabetes mellitus, methods of correction", state registration No. 0117U006000.

The scientific debate concerning optimal management of patients with arterial hypertension (AH) is still open despite effective recommendations for lifestyle modification and medicines [1]. This is connected predominantly with high incidence of complications associated with AH, notably stroke and cardiovascular diseases [2]. Furthermore, risk of complications increases drastically in patients with metabolic disorders [3]. For instance, it was reported a 2-fold increase in cardiovascular outcomes and 1.5-fold increase in all-cause mortality [2].

Variety methods for correction of metabolic dysfunction and associated disorders exist [4]. The effectiveness of carbohydrate-restricted diets was showed in numerous studies [5]. However, the data of carbohydrate-restricted diets impact on hemostasis is still insufficient, although the crucial role of the latter in pathogenesis of AH complications and metabolic disorders is undoubted [6].

In our study we investigated parameters of vascular hemostasis and coagulation in patients with basic treatment and additional carbohydrate-restricted diet.

The purpose of our study was to investigate the influence of a carbohydrate-restricted diet up to 250 g per day for 12 weeks on body mass index (BMI), insulin level, and parameters of vascular hemostasis and coagulation in patients with combination of AH, increased BMI and hyperinsulinemia, and to assess impact of this diet on course of disease.

Material and methods. The present study included 50 patients aged between 27 and 64 years old with AH, increased BMI and hyperinsulinemia (29 females and 21 males). This population was divided into two groups. 26 patients of group I (control group) received standard antihypertensive therapy, they were recommended to follow healthy life style and dietary habits. 24 patients of group II (main group) received a diet with carbohydrate restriction up to 250 g per day for 12 weeks in addition to standard therapy and healthy life style. The content of carbohydrates in diet was controlled by carbohydrate counting tables. Daily caloric intake was compensated with higher fats and proteins consumption. Standard therapy depending on peculiarities of patients included angiotensin converting enzyme inhibitor (ACEi) or angiotensin II receptors blocker (ARB), sometimes with diuretic or ACEi with calcium channels blocker (CCB) or ARB with CCB, beta-adrenergic blocker, statin and antiaggregant.

The effectiveness of therapy was controlled by measurement of day-time office BP.

AH was diagnosed according to the current guidelines [1]. 11 patients suffered from stable angina (SA) functional class (FC) I-II. 4 patients had DM II. The patients included into the research had been under observation for the period of 12 weeks.

BMI was calculated with metric formula as measured weight in kilograms divided by height in meters squared. WHO criteria were used to define nutritional status of patients.

BP measurements were obtained following a standard protocol.

We used vWF:CBA ELISA Kit to measure the binding of factor von Willebrand (vWF) to collagen and high molecular weight multimers of vWF according to the manufacturer's instructions (Technoclone, Austria).

Basal level of insulin in blood was measured using standard kit "RIO-INS-PG-125I" by radioimmunoassay. Glucose level was determined in blood using glucose oxidase.

Soluble fibrin (SF) quantification in human blood plasma was performed by double-sandwich ELISA. Monoclonal antibody FnI-3C was proposed to use as a "catch"-site in ELISA by team of investigators from the Palladin Institute of Biochemistry of the National Academy of Sciences of Ukraine.

Platelet aggregation (PA) by various inductors was studied using the Born spectrophotometric method at aggregometer "Thromlite". Such inductors of aggregation as adenosine 5-diphosphate (ADP) disodium salt in concentration $2.0 \cdot 10^6$ M ("Sigma-Aldrich"/"Merck", Canada), collagen in dilution 1:2, ristocetin in concentration 0.8 mg/ml were used.

Blood samples collections as well as measurement of BMI and blood pressure (BP) were provided twice. For the first time, data was collected immediately after recruitment the patient into the study. Once more, data was collected after 12 weeks of standard therapy (I group) or standard therapy in combination with carbohydrate restricted diet (II group).

Statistical analyses were done using "Stata-12". Numerical variables were presented as mean±standard deviation (SD) for normal type of distribution. Student t-test was used for comparisons of independent samples. Paired t-test was used for paired samples. P value < 0.05 was considered statistically significant. Categorical variables are presented as absolute numbers and percentages.

Results of the study and their discussion. Baseline characteristics of patients are shown in table 1. The results have shown no significant difference in the characteristics of patients of both groups under investigation.

Table 1

Baseline demographics, clinical and biochemical characteristics

Characteristic	Group I (n=26)	Group II (n=24)	P value
Age, Years	62.5±4.3	59.4±3.9	0.46
Male, % (n. / total)	42 (11/26)	41 (10/24)	0.54
History of AH, Years	5.5±2.5	4.7±2.2	0.34
BMI, kg/m ²	29.1±2.8	29.9±2.5	0.59
History of SA FC II-III, % (n./total)	23 (6/26)	21 (5/24)	0.52
History of DM II, % (n./total)	8 (2/26)	8 (2/24)	0.49
SBP, mmHg	164.2±3.2	165.6±4.4	0.44
DBP, mmHg	97.4±5.2	99.2±5.3	0.42
Fasting blood glucose, mmol/l	5.5±0.3	5.4±0.4	0.39
Basal insulin, mmol/l	266.2±32	276.1±35	0.23
vWF, IU/l	0.65±0.17	0.71±0.19	0.19
PA-ADP, %	33.2±4.4	31.3±3.5	0.37
PA-collagen, %	40.7±3.9	43.2±4.7	0.33
PA-ristocetin, %	32.3±2.2	30.6±3.1	0.29
SF, mcg/ml	1.56±0.4	1.62±0.36	0.23

Statistically significant trends of BP normalization were in both groups after course of treatments (table 2).

Table 2

Trends in blood pressure in research groups

Characteristic	Group I (n=26)			Group II (n=24)		
	Before treatment	After treatment	P value	Before treatment	After treatment	P value
SBP, mmHg	164.2±3.2	141.4±3.6	0.0021	165.6±4.4	139.6±3.5	0.0011
DBP, mmHg	97.4±5.2	85.9±2.6	0.0048	99.2±5.3	84.6±2.2	0.0059

Dynamics of BMI, level of fasting blood glucose, level of basal insulin in patients of both groups before and after treatment are shown in table 3. There were no statistically significant changes of BMI in both research groups. Decrease in basal insulin level by 27.8% was showed in group II after following of diet with carbohydrate restriction.

Table 3

Dynamics of BMI, fasting blood glucose, basal insulin n research groups

Parameter	Group I (n=26)			Group II (n=24)		
	Before treatment	After treatment	P value	Before treatment	After treatment	P value
BMI, kg/m ²	29.1±2.8	28.3±2.5	0.47	29.9±2.5	27.4±0.89	0.16
Fasting blood glucose, mmol/l	5.5±0.3	5.2±0.2	0.38	5.4±0.4	4.6±0.2	0.11
Basal insulin, mmol/l	266.2±32.1	242±25.4	0.14	276.1±35.5	199±15.5	0.03

It was found that following the diet with carbohydrate restriction up to 250 g daily for 12 weeks changed some parameters of hemostasis (table 4). It was revealed decrease in level of vWF after treatment course (0.32±0.13 IU/ml vs 0.71±0.19 IU/ml. p=0.009), whereas there were no statistically significant changes of vWF in group I. Statistically significant improvement of PA-ADP after course of treatment was noticed in both groups (41.4±2.1% vs 33.2±3.4% and 40.5±3.4% vs 31.3±3.5%, p=0.02 and p=0.01, respectively), while no significant changes were in PA-collagen. It was found statistically significant normalization of AP-ristocetin as well as decrease in SF only in group II.

Table 4

Trends of hemostasis before and after treatment in research groups

Parameter	Group I (n=26)			Group II (n=24)		
	Before treatment	After treatment	P value	Before treatment	After treatment	P value
vWF, IU/l	0.65±0.17	0.45±0.1	0.13	0.71±0.19	0.32±0.13	0.009
PA-ADP, %	33.2±3.4	41.4±2.1	0.02	31.3±3.5	40.5±3.4	0.01
PA-collagen, %	40.7±3.9	42.3±4.6	0.16	43.2±4.7	45.5±4.6	0.15
PA-ristocetin, %	32.3±2.2	33.5±3.1	0.36	30.6±3.1	37.4±2.1	0.01
SF, mcg/ml	1.26±0.4	1.03±0.3	0.28	1.32±0.36	0.77±0.21	0.03

Most studies dedicated to the carbohydrate-restricted diet explore its input on carbohydrates and lipids metabolism, weight, blood pressure. A carbohydrate-restricted diet is recommended not only for the management of the patients with diabetes mellitus but also for the treatment and prevention of prediabetes, obesity [5].

Thus, the decline in basal insulin level leads to the improvement of insulin influence on the endothelium. It is well-known that insulin as a hormone stimulates the activity of the sympathetic nervous system, cardiac output, BP [8], and has direct atherogenic activity: cause set of hemodynamic and metabolic changes. Such alterations may provoke complications as a result of coagulation disorders [6]. Consequently, the correction of the basal insulin level plays a key role in the prevention and slowing-down of atherosclerosis and its complications [10]. Bando H. et al. registered a remarkable decrease in preprandial and postprandial glucose levels in patients following a low carbohydrate diet which suggests the hypoglycemic effect of a carbohydrate-restricted diet. However, we haven't registered a significant decrease in fasting glucose levels. This fact may be connected with the observation that the higher glucose level is the more intensive glucose decreasing effect of carbohydrate-restricted diet is [11].

Meng Y. et al. in the meta-analysis reported the more intensive reduction in weight in patients with diabetes mellitus following a low-carbohydrate diet [12]. In our research, we have registered no significant decrease in BMI in group 2 which may be connected with a limited number of patients as well as the time of observation. However, the trend of its reduction was higher in patients following a carbohydrate-

restricted diet than the standard one. It must be added that Kelly T. et al. in the review suggests studies devoted to diets effect have some potential for bias, residual confounding, and type 1 errors [13].

Though the nutrients influence all three components of the hemostasis to a great extent [14] the data regarding carbohydrate-restricted diet is limited as mostly investigators pay more attention to the unhealthy properties of high-fat diet [5], advantages of the Mediterranean diet and DASH-diet or effects of specific products regarding hemostasis [15].

VWF is a marker of endothelium activation. VWF becomes activated after endothelium damage and the detection of endothelial cell adhesion molecules on the surface of endotheliocytes. VWF facilitates platelet adhesion to vessel wall in the damaged place as the bridge between subendothelial structures of damaged vessel wall and platelets as well as interplatelets bridge. Thrombocyte becomes activated after adhesion, gain the ability to bind vWF, fibrinogen with further promotion of platelets activation process. Thus, vWF stimulates thrombogenesis [9].

The platelets aggregation inductors (ADP, collagen) affect platelets intensively, change its functional ability. As a result, the risk of vascular complications is extremely elevated [15].

VWF activity was decreased and platelet aggregation with ADP and ristocetin was improved significantly in patients of group II (after carbohydrate restriction diet within 12 weeks), whereas the only positive trend in ADP-induced platelets aggregation was registered in group I.

Probably, a decline in vWF activity is connected with normalization of endothelial function after a decrease in basal insulin level and its impact on the endothelium [7]. It was demonstrated a positive influence of additional treatment with carbohydrate restriction on platelet aggregation which stabilizes vascular hemostasis [9].

The decrease in the SF level was registered after treatment in group II. SF represents the appearance of thrombin in the bloodstream which means activation of thrombogenic activity of blood. The decline in SF in patients of group II reflects the general reduction of coagulation abilities of blood and activation of fibrinolysis.

The main study limitation is rather small number of patients. However, this fact is connected with the relatively small compliance of patients to the following of the diet with carbohydrate restriction. Consequently, some of them were excluded from the study.

Conclusions

1. A carbohydrate-restricted diet up to 250 g per day within 12 weeks helps to decrease BMI and basal level of insulin.
2. Such changes as normalization of vWF, PA-ADP, PA-ristocetin, SF reflects the process of vascular hemostasis stabilization and, as a consequence, high effectiveness of such therapy.
3. A carbohydrate-restricted diet should be recommended to the patients with AH in combination with increased BMI and hyperinsulinemia.

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Реферати

ДІЄТИЧНА КОРЕКЦІЯ ГІПЕРІНСУЛІНЕМІЇ, ПОКАЗНИКІВ ГЕМОСТАЗУ У ХВОРИХ НА АРТЕРІАЛЬНУ ГІПЕРТЕНЗІЮ З ПІДВИЩЕНОЮ МАСОЮ ТІЛА

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Метою дослідження було вивчити вплив дієти з обмеженням вуглеводів до 250 г на добу впродовж 12 тижнів на індекс маси тіла, рівень інсуліну, показники гемостазу у хворих на артеріальну гіпертензію з метаболічними розладами. Терапія з обмеженням вуглеводів дала змогу у хворих основної групи знизити ІМТ на 7%, рівень інсуліну на 27.8%, достовірно покращити АДФ-індуковану та ристоцетин-індуковану агрегацію тромбоцитів, знизити активність фактора фон Виллебранда ($p=0.009$) і вміст розчинного фібрину ($p=0.03$). Доцільно рекомендувати гіповуглеводну дієту з обмеженням вуглеводів до 250 г на добу хворим на артеріальну гіпертензію з підвищеною масою тіла та гіперінсулінемією разом з антигіпертензивною терапією.

Ключові слова: артеріальна гіпертензія, гіповуглеводна дієта, індекс маси тіла, рівень інсуліну, показники гемостазу.

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ДИЄТИЧЕСКАЯ КОРЕКЦИЯ ГИПЕРИНСУЛИНЕМИИ, ПОКАЗАТЕЛЕЙ ГЕМОСТАЗА У БОЛЬНЫХ АРТЕРИАЛЬНОЙ ГИПЕРТЕНЗИЕЙ С ПОВЫШЕННОЙ МАССОЙ ТЕЛА

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Целью исследования было изучить влияние диеты с ограничением углеводов до 250 г в сутки в течение 12 недель на индекс массы тела, уровень инсулина, показатели гемостаза у больных АГ с метаболическими нарушениями. Проводимая терапия у больных основной группы дала возможность снизить индекс массы тела на 7%, уровень инсулина на 27.8%, достоверно улучшить АДФ-индуцированную и ристоцетин-индуцированную агрегацию тромбоцитов, снизить активность фактора фон Виллебранда ($p=0.009$) и содержание растворимого фибрина ($p=0.03$). Гипоуглеводная диета с ограничением углеводов до 250 г в сутки должна рекомендоваться больным АГ с повышенной массой тела и гиперинсулинемией наряду с антигипертензивной терапией.

Ключевые слова: артериальная гипертензия, гипоуглеводная диета, индекс массы тела, уровень инсулина, показатели гемостаза.

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THE EFFECT OF THE PSYCHO-EMOTIONAL STRESS ON THE STATE OF MICROBIOTA OF THE GINGIVAL SULCUS

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This study aimed to investigate the effect of psycho-emotional stress on the state of microbiota of the gingival sulcus in the state of eubiosis and dysbiosis in young people. The research involved 182 students of the medical university. As a model of acute psycho-emotional stress the situation of passing an important exam was used. A microbiological examination of the total microbial population of gingival sulcus and its population of microbiota, was performed with the standard methods. The impact of the stressor on the background of imbalance of the microbial homeostasis of the tooth-gingival crevice, causes the intensification of the imbalance of microbial associations in the form of commensal microflora reduction and the increase of opportunistic microflora.

Keywords: microbiota of the gingival sulcus, eubiosis, dysbiosis, psycho-emotional stress.

The work is a fragment of the research project "Study of the role of opportunistic and pathogenic infectious agents with different sensitivity to antimicrobial antiviral drugs in human pathology», state registration No. 0118U004456.

The complex of symbiotic microorganisms, that inhabit the open biotope of a macroorganism, make up its microbiome, provide a colonization resistance and have a great impact on the health and development of pathological processes in the human body. [4, 5, 8, 9].

The oral cavity has one of the most diverse microbiome in the human organism, which includes bacteria, fungi, protozoa and viruses. The uniqueness of the biotope of the gingival sulcus lies in the fact that quantitative and qualitative changes of microbial communities (dysbiosis) of this micro-ecological system can lead to major dental diseases such as: gingivitis, periodontitis and caries. Structural and

functional disorders of the microbiota of the gingival sulcus lead to pathological changes that have not only local but also systemic significance [15].

The impact of psycho-emotional stress on the microbiome of the body has been studied by the vast majority of researchers on the example of the intestinal microbiota. It is shown that the stress factors contribute to the imbalance of the intestinal microbiota and enhance the growth of pathogenic organisms [6, 7, 10, 12]. A limited number of studies are dedicated to the influence of psycho-emotional stress on the microbiota of the oral cavity [13, 14], and the state of microbiota of gingival sulcus has not been yet investigated. It is important to examine the extent of the effects of psycho-emotional factors in the areas of the oral cavity, which are responsible for the development of the most common dental diseases such as inflammatory periodontal diseases and caries, namely, biotope of a gingival sulcus. Studying the changes of microbiota of the gingival sulcus under different micro-ecological situations in the oral cavity will permit to better analyze interaction between bacteria and the immune response under the conditions of the body's stress reactions.

The purpose of the work was to study the effect of psycho-emotional stress on the state of microbiota of the gingival sulcus that is in the state of eubiosis and dysbiosis, in young people.

Materials and methods. The research involved 182 students of the medical university at the age of 19-29 years without somatic diseases. Group 1 consisted of 22 people (11 men, 11 women), in which clinically no lesions of periodontal tissues and teeth were detected. Group 2 was formed of 57 people (29 men, 28 women), who were diagnosed with chronic catarrhal gingivitis and carious lesions of dental hard tissues. As a model of acute psycho-emotional stress, a natural situation was used of passing an important exam socially significant in the lives of the young people. The survey was conducted just before the exam, namely in the state of psycho-emotional tension. As control indicators, those indicators were used, which were obtained in the state of relative calm, that is during normal college classes in the middle of the semester. To determine the level of personal and reactive anxiety the scale of C.D. Spielberger was used and adapted by J.L. Khanin. An index that is less than 30 points indicates low anxiety, from 30 to 45 points - moderate, above 45 points - high [3].

The microbiological examination of the total microbial population of the gingival sulcus and its population of individual species of microbiota was performed on the methods, which are based on anaerobic and aerobic cultivation. The taking of the content sample of the gingival sulcus was conducted in the morning on an empty stomach, with the help of a sterile paper endodontic pin of standard size (№30), of 1 cm length, which after impregnation was put in the sterile saline solution and carefully washed. The usage of standard dilution was conducted on the special, selective and differential diagnostic culture mediums: blood agar, yolk-salt agar, Sabouraud medium, Endo's medium, sugar agar with the further cultivation under aerobic and anaerobic conditions. On the samples which were obtained in aerobic cultivation conditions, the microbial population of gingival fluid of aerobic and facultative anaerobic bacteria was determined (further conventionally called aerobes). On the samples under the anaerobic cultivation conditions using our proposed method (Patent №62889, Ukraine) [3] the microbial population of facultative and obligate anaerobes was determined (further conventionally called anaerobes). An identification of the isolated pure cultures was carried out by morphological, tinctorial, cultural and biochemical characteristics. The results of quantitative research were expressed through the decimal logarithm by colony forming units per 1 ml – lg CFU / ml. There was also determined the frequency of detection of some representatives of the colonizing microflora in people of the studied groups.

All the patient investigations conformed to the principles outlined in the Declaration of Helsinki and have been performed with the permission N123, June 2015, released by the responsible Ethic's Committee of Ukrainian Medical Stomatological Academy. All the patients were informed about the purposes of the study and have signed their "consent of the patient". This article does not contain any studies with animals performed by any of the authors. The statistical analysis of survey results was implemented with the help of the programs SPSS 17.0 and Microsoft Excel 2003 [4].

Table 1

Impact of psycho-emotional tension on total microbial colonization of the gingival sulcus, lg CFU/ml (M ± m)

Indices	Intact gums and teeth		Gingivitis and caries	
	State of relative calm	Stress	State of relative calm	Stress
Colonization by aerobes	7.26±0.04	7.40±0.03 [^]	7.79±0.03*	7.85±0.03*
Colonization by anaerobes	6.81±0.04	7.07±0.08 [^]	7.73±0.03*	7.76±0.03*

Note: * - the likelihood of differences of indexes in people with gingivitis and caries compared with a group of people without lesions of teeth and gums by Student's test, $p < 0.05$; [^] - the likelihood of indexes difference in people under conditions of emotional stress in comparison with the state of relative calm by Student's test, $p < 0.05$.

Results of the study and their discussion. In the first phase of the study the characteristic condition of the microbiome of the gingival sulcus was defined (eubiosis or dysbiosis) in young people with different dental status in the state of relative calm.

As it is shown in table 1, quantitative and qualitative composition of a gingival sulcus microbiota among young people with gingivitis and caries was different from those with intact teeth and gums. In the state of relative calm the microbial load of the biofilm of the gingival sulcus in patients of the second group was higher than those of the first group. As our research has shown, the population of the gingival sulcus of aerobic and anaerobic microflora in people with gingivitis and caries in the state of relative calm was exceeding the indicators in young people with intact teeth and gums by 3, 4 times ($p<0.05$) and in 8.3 times ($p<0.05$), respectively.

The qualitative composition of the microbiota of the gingival sulcus in the state of relative calm also depended on the dental status.

Table 2

Impact of psycho-emotional tension on the composition of microbiota of the gingival sulcus in people without lesions of periodontal tissues and teeth, % people/lg CFU/ml ($M \pm m$)

Microorganisms	State of relative calm	Stress
S.viridans spp.	95.5/6.91±0.09	81.8/6.79±0.05
S.γ-haemolyticus spp.	81.8/6.81±0.10	95.5/7.08±0.06
S.β-haemolyticus spp.	9.1/5.80±0.30	9.1/6.51±0.39
Neisseria spp.	36.4/5.88±0.26	36.4/7.07±0.10 [^]
Corynebacterium spp.	31.8/5.84±0.25	4.5 [^] /5.30
Lactobacillus spp.	27.3/5.30±0.10	9.1 [^] /5.25±0.25
S.epidermidis	31.8/4.99±0.13	31.8/5.65±0.23 [^]
Bacillus spp.	18.2/5.28±0.19	9.1/5.50±0.20
Actinomyces spp.	0/0	0/0
S.aureus	0/0	0/0
Enterobacteriaceae	0/0	0/0
Candida spp.	0/0	0/0

Note: [^] – the likelihood of frequency difference by φ Fisher criterion and lg CFU/ml in the study groups by Student's criterion under conditions of psycho-emotional tension in comparison with the state of relative calm, $p<0.05$.

Table 3

Impact of psycho-emotional tension on the composition of microbiota of the gingival sulcus in people with catarrhal gingivitis and caries % people/ lg CFU/ml ($M \pm m$)

Microorganisms	State of relative calm	Stress
S.viridans spp.	31.6 ["] /6.43±0.14 [*]	12.3 ["] [^] /6.74±0.14
S.γ-haemolyticus spp.	89.5/7.35±0.05 [*]	96.5/7.34±0.04 [*]
S.β-haemolyticus spp.	28.1 ["] /6.39±0.21	21.1/6.68±0.20
Neisseria spp.	73.7 ["] /7.33±0.08 [*]	89.5 ["] [^] /7.62±0.04 [*] [^]
Corynebacterium spp.	19.3/5.24±0.21	1.8 [^] /5.7
Lactobacillus spp.	8.7 ["] /4.86±0.22	5.3/5.43±0.29
S.epidermidis	42.1/5.50±0.19 [*]	22.8 [^] /5.47±0.29
Bacillus spp.	38.6 ["] /5.58±0.22	56.1 ["] /6.40±0.13 [*] [^]
Actinomyces spp.	8.8 ["] /5.24±0.10	8.8 ["] /5.82±0.48
S.aureus	21.1 ["] /5.0±0.20	26.3 ["] /5.02±0.13
Enterobacteriaceae	26.3 ["] /5.12±0.16	42.1 ["] [^] /5.36±0.10
Candida spp.	26.3 ["] /4.61±0.10	28.1 ["] /4.66±0.13

Note: * - the likelihood of differences lg CFU/ml of people with gingivitis and caries compared with a group of people without lesions of teeth and gums by Student's criterion, $p<0.05$; ["] – the likelihood of frequency difference of detection of certain microorganisms in groups of people with gingivitis and caries in comparison with a group of people without lesions of teeth and gums by φ Fisher criterion, $p<0.05$; [^] – the likelihood of frequency difference by φ Fisher criterion and lg CFU/ml groups by Student's criterion under conditions of psycho-emotional tension compared with the state of relative calm, $p<0.05$.

Thus, tables 2.3 proved, that the frequency of colonization of the gingival sulcus by alpha hemolytic streptococcus in people with gingivitis and caries was at 63.9% ($p<0.05$) lower than in people without dental and gums diseases. Also, under conditions of development of dental diseases in 3.0 times ($p<0.05$) decreased the population density of the gingival sulcus by S.viridans spp .

There was a downward trend of the frequency of colonization of Corynebacterium spp. in people with gingivitis and caries, although these changes were not reliable. The density of population of the gingival sulcus by γ-hemolytic streptococci, which include S.mutans in patients with dental and gums diseases was 3.5 times($p<0.05$) higher, than in those without this pathology. Neisseria spp. by 37.3% was detected more often in people with gingivitis and caries, also the colonization density by Neisseria spp. was higher in this examined group at 28.2 times ($p<0.05$), compared with the people without teeth and gums diseases.

The density of population of epidermal staphylococcus in patients with catarrhal gingivitis and caries was 3.2 ($p < 0.05$) times higher, than in those without these diseases.

In the second group of people, more often than those with intact teeth and gums opportunistic microorganisms were found: *Bacillus* spp. at 20.4% ($p < 0.05$), *S.aureus* at 21.1% ($p < 0.05$), *S.β-haemolyticus* spp. at 19.0% ($p < 0.05$), *Actinomyces* spp. at 8.8% ($p < 0.05$), Enterobacteriaceae at 26.3% ($p < 0.05$), *Candida* spp. at 26.3% ($p < 0.05$). We have found a reduction in the frequency of colonization of *Lactobacillus* spp. in patients with catarrhal gingivitis and caries at 18.6% ($p < 0.05$).

If you use the classification of dysbiosis of oral cavity by Khazanova V.V. [3], then 100% of people with intact teeth and gums in the state of relative calm had an eubiosis, and 100% of people with catarrhal gingivitis and caries had a dysbiosis (in 42.1% - was found a dysbiotic shift, 57.9% - dysbiosis of 1-2 degree). Thereby, the further research was to examine the influence of psycho-emotional stress on the characteristics of the microbiome of the gingival sulcus that is in the state of eubiosis and dysbiosis.

The objective criteria of presence of examination stress in examined people were indicators of the level of reactive anxiety. In the group of people with intact teeth and gums an increase in reactive anxiety was found at 24.6% ($p < 0.05$), in patients with catarrhal gingivitis and caries – at 14.3% ($p < 0.05$), which confirmed the presence of the stressful situation.

The study on the influence of stress agents on the state of microbiota of the gingival sulcus showed some changes in biological characteristics of the resident microflora. Under conditions of psycho-emotional tension the total microbial population of the gingival sulcus in people with eubiosis increased for aerobic microorganisms in 1.4 times ($p < 0.05$), for anaerobic- in 1.8 times ($p < 0.05$). As far as people with dysbiosis the total microbial load of the gingival sulcus didn't significantly change and remained at a high level.

Let us consider how the microbiota of the gingival sulcus changed in the state of eubiosis and dysbiosis in people who were exposed to emotional stress.

In the first group of people (at the state of relative calm an eubiotic state of microbiota was detected) *Lactobacillus* spp. plated by 18.2% less ($p < 0.05$), *Corynebacterium* spp. – by 27.3% less ($p < 0.05$). At the same time an increase of the microbial number of *S.epidermidis* was observed in 4.6 times ($p < 0.05$) and *Neisseria* spp. in 15.4 times ($p < 0.05$).

In the people who were in the state of relative calm a dysbiotic state of the microbiota (2nd group) was detected, by 19.3% less ($p < 0.05$) was plated *S.viridans* spp., by 17.5% ($p < 0.05$) - *Corynebacterium* spp., by 19.3% ($p < 0.05$) - *S.epidermidis*, often was found *Neisseria* spp. (by 15.8%, $p < 0.05$) and Enterobacteriaceae (by 15.8%, $p < 0.05$), increased the density of colonization of *Neisseria* spp. in 1.9 times ($p < 0.05$), *Bacillus* spp. – in 6.6 times ($p < 0.05$).

Describing the changes in microbiota of the tooth-gingival crevice in the state of psycho-emotional stress, it should be noted that, in 2 students out of the first group of 22 people, the emergence of imbalances of microbial populations were detected, which were characterized as a dysbiotic shift. In all the people of the second group who were involved in the action of stressful factors on the background of existing dysbiotic changes of microbiota, an increasing imbalance of microbial associations was observed. Among the 57 of the examined people, the dysbiotic shift was found in 18 people (31.6%), dysbiosis of 1-2 degree – 39 people (68.4%).

Thereby, our study showed that, psycho-emotional stress had an influence on the quantitative and qualitative characteristics of the gingival sulcus microbiota of both examined groups, however this effect was more significant in the second group. The microbiota, which already has an imbalance of microbial populations on the action of the stress factor, reacted with more intensive violation of the correlation of symbiotic and opportunistic microflora [12]. The frequency of population of *S.viridans* spp. in people with dysbiosis of the biofilm of the gingival sulcus was lower at 69.5% ($p < 0.05$) compared with the people with eubiosis. The second group of examined people were more likely to have the opportunistic microorganisms *Bacillus* spp., *Actinomyces* spp., *S.aureus*, Enterobacteriaceae, *Candida* spp. Most often the second group of people had : *Bacillus* spp.- at 47.0% ($p < 0.05$) more often than people of the first group, *S.aureus* – in 26.3% ($p < 0.05$) cases, *Actinomyces* spp. - in 8.8% ($p < 0.05$), Enterobacteriaceae – in 42.1% ($p < 0.05$), *Candida* spp. – in 28.1% ($p < 0.05$) cases.

We showed that the microbiota is a highly sensitive indicator system, that reacts with quantitative and qualitative changes, influenced by factors of the external and internal environment. A subjective assessment of the significance of the exam can turn it into a strong emotional stressor. Studies have shown, that psycho-emotional stress influences the state of microbiota of the gingival sulcus, although the level of this impact depends on the initial state of the balance of microbial associations. Under conditions of dysbiosis - the initial imbalance of microbial populations increases a susceptibility to invading pathogens [14].

The development of psycho-emotional tension in young people in conclusion of our data leads to a decrease of the level and frequency of colonization of the gingival sulcus by stabilizing microflora. The disbalance of representatives of the resident microflora with a shift towards the opportunistic microflora is a prerequisite for the development of infectious caused pathology of the oral cavity [15].

Consequently, the psycho-emotional tension influences the biological characteristics of a gingival sulcus microbiota. The stress-induced changes of microbiota are different in nature, depending on the initial state of the ratio of bacterial populations in the it. An eubiotic character of the interaction of microbial populations is a stabilizing factor and provides an adaptive support for quantitative and qualitative composition of microbiota. The impact of the stressor on the background of an imbalance of the microbial homeostasis of the tooth-gingival crevice, causes a strengthening imbalance of the microbial associations in the form of reducing the commensal microflora and increasing of the opportunistic microflora.

Conclusion

The impact of the stressor on the background of imbalance of the microbial homeostasis of the tooth-gingival crevice, causes the intensification of the imbalance of microbial associations in the form of commensal microflora reduction and the increase of opportunistic microflora.

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Реферати

ВПЛИВ ПСИХО-ЕМОЦІЙНОГО СТРЕСУ НА СТАН МІКРОБІОТИ ЯСНЕВОЇ БОРОЗНИ

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Це дослідження мало на меті дослідити вплив психоемоційного стресу на стан мікробіоти ясенної борозни у стані еубіозу та дисбіозу у молодих людей. У дослідженні взяли участь 182 студенти медичного університету. Як модель гострого психоемоційного стресу була використана ситуація складання важливого іспиту. Мікробіологічне дослідження загальної мікробної популяції ясенної борозни та її популяції мікробіоти проводили стандартними методами. Вплив стресового фактору на тлі дисбалансу мікробного гомеостазу зубо-ясенної щілини викликає посилення

ВЛИЯНИЕ ПСИХОЭМОЦИОНАЛЬНОГО СТРЕССА НА СОСТОЯНИЕ МИКРОБИОТЫ ДЕСНЕВОЙ БОРОЗДЫ

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Это исследование имело целью исследовать влияние психоемоционального стресса на состояние микробиоты десневой борозды в состоянии эубиоза и дисбиоза у молодых людей. В исследовании приняли участие 182 студента медицинского университета. Как модель острого психоемоционального стресса была использована ситуация сдачи важного экзамена. Микробиологическое исследование общей микробной популяции десневой борозды и ее популяции микробиоты проводили стандартными методами. Влияние стрессового фактора на фоне дисбаланса микробного гомеостаза зубо-десневой щели вызывает

дисбалансу мікробних асоціацій у вигляді зменшення коменсальної мікрофлори та збільшення умовно-патогенної мікрофлори.

Ключові слова: мікробіота ясеневі борозни, еубіоз, дисбіоз, психоемоційний стрес.

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усиление дисбаланса микробных ассоциаций в виде уменьшения коменсальной микрофлоры и увеличения условно-патогенной микрофлоры.

Ключевые слова: микробиота десневой борозды, эубиоз, дисбактериоз, психоэмоциональное напряжение.

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THE SCOPE OF INPATIENT MORTALITY DUE TO SAFETY INCIDENTS IN DOMESTIC HOSPITALS

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The problem of unintentional harm to patients is common in all countries, and especially in low- and middle-income countries. The article analyzes methodological approaches to assessing the levels of patient safety incidents in healthcare institutions. It has been established that more than 18 thousand patients die in Ukraine as a result of adverse events that can be prevented, which is significantly more than the number of victims of road traffic accidents and industrial accidents. The data obtained indicate the extreme urgency of the problem of unintentional harm to patients and the need for urgent measures to improve patient safety in domestic health care facilities. The purpose of the work was to assess patients' mortality scope due to safety incidents in domestic hospitals. Were analyzed thematic scientific publications, statistical directories of the Center for Medical Statistics of the Ministry of Health of Ukraine, national reports, and analytical reviews on the state of man-made and natural safety in Ukraine.

Keywords: incident, patient safety, health care, inpatient mortality, unintentional harm.

The work is a fragment of the research project "Science on the establishment of an optimal risk management system to ensure a safe medical environment", state registration No. 0120U101432.

During the provision of medical care, along with the benefit to the patient, for various reasons and primarily due to errors of medical personnel and other reasons that can be prevented, prerequisites for harm or harm to the patient arise, that is, patient safety incidents occur [9]. The adverse events (an incident that caused damage) caused by the unsafe treatment or patient care (organizational, diagnostic, treatment errors, traumatizing of patient in a healthcare facility as a result of a fall, injury by physical, chemical, or biological factors, etc.) is considered nowadays one of 10 [10], and in some studies [11] one of 3 leading causes of death and disability worldwide.

The terms "patient safety incident" and "adverse event" are used interchangeably in most works. In addition, phrases such as "unintentional harm" and "preventable harm" are used identically.

The problem of patient safety is extremely relevant for countries with low and middle incomes [10]. In addition to medical and social aspects, the problem of patient safety has a significant economic component. It has been established [14] that up to 15% of the funds allocated for inpatient care are spent on the treatment of patient safety cases.

Data on patient safety incidents and their consequences have prompted the international community and some countries to take large-scale measures to minimize this problem. Only in 2018–2019 were adopted four thematic declarations, two of which were initiated by the WHO [2,3]. These declarations mention, among other things, the significant potential for creating a hospital environment that is safe for patients.

As for Ukraine, there have been practically no research on prevalence estimates of patient safety cases incidents and their consequences, except in certain areas of medical practice, such as anesthesiology and intensive care [7].

The available data on the extension of unfavorable for patient health care cases are questionable.

Thus, officially according to the Y40-Y84 codes (complications due to therapeutic and surgical interventions) of the International Classification of Diseases (ICD 10) in Ukraine in 2017 were registered 1,334 deaths [1]. We have similar values for this indicator for 2018.

But there is reason to suppose that these figures are significantly underestimated [4,5], which, in our opinion, explains the underestimation of the problem of patient safety in Ukraine.

The best option for assessing the scale of the negative consequences of patient safety incidents in domestic hospitals would be to establish them by analyzing primary medical materials, and we are doing

this work. However, other approaches can be used to make a preliminary assessments of the consequences of health care defects.

Thus, it is believed [8] that the level of in-patient mortality due to errors of medical personnel exceeds the number of victims of road traffic accidents (accidents) and victims of occupational traumatism combined.

This approach is valuable because the number of victims of accidents and occupational traumatism in Ukraine is fairly accurately recorded.

In this regard, there may be interesting approaches derived from the analysis of the frequency of adverse events in 26 hospitals in eight middle-income countries [13,15]. These studies found that the level of adverse events in these countries was about 8% for hospital patients, and almost a third of them (30%) had safety incidents directly or indirectly related to their deaths. Again, in the reports of the Center for Medical Statistics of the Ministry of Health of Ukraine, one can easily find data for recent years on the number of patients treated in domestic hospitals.

Patient mortality from safety incidents can also be estimated using data from the US National Academy of Engineering and Medicine (NASEM) [12]. It is estimated that 134 million adverse events occur each year due to unsafe care in hospitals in low- and middle-income countries, resulting in 2.6 million patient deaths.

The above methodological approaches to assessing the levels of unintentional harm to patients, as well as the relationship between the number of safety incidents and the disability and patient mortality as a result, can be used for similar estimates in domestic health care.

Thus, knowledge of the extent of harm to patients is important information for the medical and patient communities, as well as an important prerequisite for making appropriate decisions to improve patient safety.

The purpose of the work was to assess patients' mortality scope due to safety incidents in domestic hospitals

Materials and Methods. Were analyzed thematic scientific publications, statistical directories of the Center for Medical Statistics of the Ministry of Health of Ukraine, national reports, and analytical reviews on the state of man-made and natural safety in Ukraine.

Research methods used in the study were bibliosemantic, statistical, mathematical. Approaches to assessing the consequences of unintended harm to patients, proposed by the Organization for Economic Co-operation and Development (OECD) in their projects [14].

Results of the study and their discussion. Based on the judgment [8] that the inpatient mortality rate due to errors of medical personnel exceeds the sum of victims of accidents and injuries at work, we found that for this reason more than 10,770 people die in domestic hospitals. This figure is the sum of the number of deaths due to the road traffic accidents in 2007 (9,589 people) and the number of fatal industrial injuries in the same year (1,181 people) in Ukraine.

2007 was adopted as the base year, as it was then that the maximum mortality rates of Ukrainian citizens as a result of road accidents were recorded. Lately, at all levels, were taken the effective measures to improve a road safety. Also, until 2007, attention to patient safety, occupational safety and health, and road safety was roughly equally insufficient. In contrast to the indicators of fatal occupational injuries and deaths from road accidents, which, according to the State Statistics Service of Ukraine, tend to decrease after 2007, the rate of inpatient mortality, as before 2007, continues to grow [6].

That is, today the mortality rate of patients due to safety incidents is possibly much higher.

Taking into account the data [8,10] and considering that in 2007 in domestic hospitals were treated 11,732,456 people, according to the Center for Medical Statistics of the Ministry of Health of Ukraine, we receive 938,600 people (8% from 11,732,456 treated in hospitals in 2007), who could be exposed to a medical error or other adverse event. Accordingly, in 30% of people, ie 281,580 out of 938,600, medical care defects were the main or indirect cause of death. If we assume that only in one of ten of these people medical care defects was the main cause of death, it is 28,158 people.

In addition, according to the NASEM data logic [12], we find that 1.94% of patients (2,600,000 patient deaths per 134,000,000 adverse events) who are caused unintentional harm, die. We have already noted above that in 2007 about 938,600 people suffered damage in Ukrainian hospitals while receiving medical care and 1.94% of this number - 18,208 people.

Summarized data on the calculation of patient mortality due to defects in medical care are given in the table. We see that the values of inpatient mortality rates, which could be avoid due to preventable reasons, obtained by different methodological approaches are quite close, and the average value exceeds 18 thousand people.

Data on patient mortality in domestic HCFs through safety incidents according to different methodological approaches and ratios

Scientific sources of methodological approaches and ratio for assessing the scale and consequences of unintentional harm to patients	Estimates of mortality in domestic CHCs due to safety incidents, persons
Baker T., 2005 [8]	> 11 951
Wilson R.M. et al., 2012 [15]	28 158
NASEM, 2018 [12]	18 208
Average	> 18 262

Measures taken at various levels have had a positive impact on the safety of road users and the safety of workers. According to the State Emergency Service of Ukraine [1], in 2017 the death rate from road accidents was 4,529 people, and as a result of industrial injuries - 366 people, that is, the mortality in these areas has decreased more than twice compared to 2007. Whereas inpatient and postoperative mortality as indicators of patient safety after 2007 tends to increase [6] due to lack of proper attention to the problem [5]. That is, the problem of patient safety due to its negative medical, social and demographic consequences is much more relevant today than fatal injuries due to road accidents and fatal occupational injuries, which require urgent measures to minimize patient safety incidents.

Conclusions

1. The problem of unintentional harm to patients is common in all countries, and especially in low- and middle-income countries.
2. Each year more than 18 thousand patients die in hospitals due to reasons that could be prevented.
3. The number of patients who die in domestic health care facilities exceeds the number of victims of road traffic accidents and victims of occupational traumatism combined.
4. The problem of patient safety in Ukraine, given its relevance, requires in-depth research and priority attention from the state authorities to minimize it.

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Реферати

**МАСШТАБИ СТАЦИОНАРНОЇ ЛЕТАЛЬНОСТІ
ПАЦІЄНТІВ ЧЕРЕЗ ІНЦИДЕНТИ
БЕЗПЕКИ У ВІТЧИЗНЯНИХ ЛІКАРНЯНИХ
ЗАКЛАДАХ**

Науменко О.М., Скалецький Ю.М., Риган М.М.,
Дідковський В.Л.

Проблема ненавмисної шкоди пацієнтам властива для всіх країн, і особливо для країн з низьким і середнім рівнем доходів населення. У статті проаналізовано методичні підходи до оцінки рівнів інцидентів безпеки пацієнтів у закладах охорони здоров'я. Установлено, що внаслідок несприятливих подій, які можна попередити, в Україні помирає більше 18 тис. пацієнтів, що значно більше, ніж кількість жертв унаслідок дорожньо-транспортних пригод і нещасних випадків на виробництві. Отримані дані свідчать про надзвичайну актуальність проблеми ненавмисної шкоди пацієнтам і потребу в невідкладних заходах з покращення безпеки пацієнтів у вітчизняних закладах охорони здоров'я. Метою роботи була оцінка масштабів летальності пацієнтів через інциденти безпеки у вітчизняних лікарняних закладах. Під час підготовки публікації аналізувалися тематичні наукові публікації, статистичні довідники Центру медичної статистики МОЗ України, національні доповіді та аналітичні огляди про стан техногенної та природної безпеки в Україні.

Ключові слова: інцидент, безпека пацієнтів, охорона здоров'я, стаціонарна летальність, ненавмисна шкода.

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**МАСШТАБИ СТАЦИОНАРНОЙ
ЛЕТАЛЬНОСТИ ПАЦИЕНТОВ ИЗ-ЗА ИНЦИДЕНТОВ
БЕЗОПАСНОСТИ В ОТЕЧЕСТВЕННЫХ ЛЕЧЕБНЫХ
УЧРЕЖДЕНИЯХ**

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Дидковский В.Л.

Проблема непреднамеренной вреда пациентам свойственна для всех стран, и особенно для стран с низким и средним уровнем доходов населения. В статье проанализированы методические подходы к оценке уровней инцидентов безопасности пациентов в учреждениях здравоохранения. Установлено, что в результате неблагоприятных событий, которые можно предупредить, в Украине умирает более 18 тыс. пациентов, что значительно больше, чем количество жертв в результате дорожно-транспортных происшествий и несчастных случаев на производстве. Полученные данные свидетельствуют о чрезвычайной актуальности проблемы непреднамеренного вреда пациентам и потребность в неотложных мероприятиях по улучшению безопасности пациентов в отечественных учреждениях. Цель работы - оценка масштабов летальности пациентов из-за инцидентов безопасности в отечественных лечебных учреждениях. При подготовке публикации анализировались тематические научные публикации, статистические справочники Центра медицинской статистики Минздрава Украины, национальные доклады и аналитические обзоры о состоянии техногенной и природной безопасности в Украине.

Ключевые слова: инцидент, безопасность пациентов, здравоохранение, стационарная летальность, непреднамеренный вред.

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**ASSESSMENT OF THE FUNCTIONAL TYPES OF BODY MOBILIZATION BASED
ON A DYNAMIC ANALYSIS OF SPECTRAL INDICATORS OF HEART RATE VARIABILITY
AND THEIR CLASSIFICATION**

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The article is dedicated to the blessed memory of the hrv method and the cybernetic model of dual-circuit regulation of cardiac activity author Roman Markovich Baevsky (August 3, 1928 - May 31, 2020.)

The article is devoted to the study of electromagnetic phenomena of human cardiac activity and the possibilities of their clinical use in practical medicine to assess the level of health in order to prevent NCDs. The results of a dynamic analysis of the spectral parameters of cardiac activity during the performance of an orthostatic test by functionally healthy people are given in the article. The authors described four functional types of mobilization/adaptation, gave a characterization and interpretation to them, proposed a variant of their classification based on the cybernetic model of dual-circuit regulation of cardiac activity R.M. Baevsky.

Key words: heart rate variability, spectral analysis.

The work is a fragment of the research project "Development of algorithms and technology for introducing a healthy lifestyle in patients with non-communicable diseases based on the study of psycho-emotional status", state registration No. 0116U007798.

Studying the processes of mobilization/adaptation, signaling continues to be one of the fundamental areas of systems biology and systems medicine. Adaptation provides daily adaptation to the action of the external environment and is of key etiological importance in the etiopathogenesis of non-communicable diseases (NCDs). Electromagnetic phenomena play a fundamental role in the functioning of the human body, and they should continue to be studied to further solve the global problem of NCDs.

The electromagnetic phenomena of cardiac activity can and should be considered as a sensitive indicator of the adaptive reactions of the whole organism because they have great prognostic and diagnostic

potential. The advent of the possibility of spectral analysis of cardiac activity frequencies has made the method of heart rate variability (HRV) the most accessible for studying the electromagnetic phenomena of the human body in clinical practice. However, the HRV method is not used for this purpose by doctors due to the lack of clear clinical recommendations and a systematic understanding of this issue. [2, 3, 7, 10, 11].

The purpose of the work was to help solve the problem of NCDs by improving diagnostics based on the study of electromagnetic phenomena in the body using modern high technology.

Materials and methods. The results of an open, non-randomized, controlled study of 82 functionally healthy people were taken to develop methods for the dynamic analysis of spectral parameters of cardiac activity in HRV. 60 athletes (n1 group, average age – 20,5±4,7 years, men – 100%;) and 23 anamnesticly functionally healthy doctors studied at the faculty of postgraduate education and who did not engage in professional sports (n2 group, average age - 24,8±2,0 years, men – 78%; control) were examined [12]. Statistical analysis was performed on 72 HRV-short record results. 10 records were excluded from the analysis for technical reasons. The analyzed record consisted of two parts: 1) background (BG) recording (duration 5 minutes) and orthostatic test (OT) recording (duration 3 minutes). The analyzed records were obtained in compliance with all technical requirements, using certified equipment (model Poly-Spectr, Neurosoft Company, Ivanovo, Russia), in compliance with ethical standards [12]. Dynamic data analysis with the construction of graphical material, the classification of the results were performed in accordance with the hypothesis of the authors [1, 3]. Indicators Total power (TP, mc²), Very low frequency (VLF, mc², %; 0,0033 – 0,04 Hz), Low Frequency power (LF, mc², %; 0,04 – 0,15 Hz), High Frequency power (HF, mc², %; 0,15 – 0,4 Hz), a ratio of Low Frequency to High Frequency LF/HF, the normalized (or normalized unit) spectral indices LFnu and HFnu (%) were presented in as average values with their average error (M±m) and were estimated with the construction of the diagram as well. Statistical analysis was performed using the Prism 5.0 software package. One-way analysis of variance) was used to test the null hypothesis in comparing multiple groups. Tukey's Multiple Comparison Test Tukey's honestly significant difference test (Tukey's HSD test) was used to refine the values of many groups.

Results of the study and their discussion. A different mobilization reaction to the orthostatic test among functionally healthy respondents and its regularities were established: a visual analysis of the graphic dynamics of the spectral indicators of cardiac activity revealed four repeatability options A, B, C, D. The presence of clear patterns of the dynamics of spectral indicators for each A-, B-, C-, D- options for mobilization during the orthostatic test were established and functionally healthy respondents were divided into groups according to types (table 1).

Table 1

Groups (options) of the heart rhythm spectral parameters dynamics after the orthostatic test in functionally healthy people

	Group 1 Type A n=18(25%) n1=11(61%) n2=7(39%)	Group 2 Type B n=20(28%) n1=14 (70%) n2=6 (30%)	Group 3 Type C n=25(35%) n1=20 (80%) n2=5 (20%)	Group 4 Type D n=9(12%) n1=6 (67%) n2=3 (33%)
Age, year	21.78±4.01	24.00±5.29	19.00±4.31	23.00±8.77
TP, mc ²	↓ or ↑(33%)	↓ or ↑(70%)	↓(100%)	↑(100%)
VLF, mc ²	↓(100%)	↑(100%)	↓(100%)	↑(100%)
LF, mc ²	↑(100%)	↓(70%) or ↑	↓(100%)	↑(100%)
HF, mc ²	↓(100%)	↓(100%)	↓(100%)	↓ or ↑(78%)
VLF, %	↓(89%) or ↑	↑(100%)	↓ or ↑(88%)	↓ or ↑(56%)
LF, %	↑(100%)	↓(90%) or ↑	↓(92%) or ↑	↓ or ↑(89%)
HF, %	↓(100%)	↓(100%)	↓(100%)	↓(100%)

The numerical indices values of HRV spectral analysis are presented in table 2.

Testing the null hypothesis confirmed the presence of a number of significant differences in a number of compared indicators of the spectrum of cardiac activity in the compared groups of types of mobilization of functionally healthy individuals. This was confirmed using the Tukey criterion as well.

It was established that a change in the numerical indicators of the frequency spectrum of cardiac activity logically corresponds to the graphic dynamics in the selected groups. The patterns of randomization depending on sports were not established: an approximately equal distribution of athletes and non-athletes was recorded for each type of response. The following specific characteristics of the types of mobilization were established (table 1, 2):

1) type A was characterized by a reaction of an increase in the absolute and relative values of LF with a decrease in the absolute and relative values of HF, VLF; The VLFBG value was greatest with this type A, and the VLFOT value was greatest with this type A as well;

2) type B was established by the type of mobilization due to the growth of VLF with a decrease in the absolute and relative contributions of other frequencies of the cardiac spectrum;

3) type C was characterized by a decrease in the entire frequency spectrum during mobilization; an increase in the relative VLF was characteristic of most respondents with type C; TCBG level was set the highest in the group of respondents with type C;

4) type D was characterized by an increase in all absolute values of spectral values with a change in the proportions of relative values: a decrease in the share of HF and an increase in the shares of VLF, LF were found in respondents with type D mobilization; these respondents had the lowest values of all indicators (TC, VLF, HLF, LF, HF) in the background before the OT.

Table 2

Comparative characteristics of the spectral analysis parameters of heart rhythm after an orthostatic test in functionally healthy people

	Group 1 Type A	Group 2 Type B	Group 3 Type C	Group 4 Type D
R-RBG, bpm	60.5±23.41	56.95±14.72	60.5±11.58	56.00±13.91
R-ROT, bpm	85.00±22.82	79.30±20.33	84.40±13.15	75.80±13.14
TPBG, mc2	3079±6947	3843±43.66	6640±12443***	1124±588***
TPOT, mc2	4183±7863	4830±10104**	1699±1563**	5886±4381
VLFBG, mc2	1401±3045*	1112±757.6*	1791±3326***	437.0±226.8***
VLFOT, mc2	829±2031	2178±2139**	732.0±733,11**, *	1966±2824*
LFBG, mc2	975.5±954.9	1138±2684	2063±3508*	337±313.5*
LFOT, mc2	2281±3846	1807±5304**	833±1018**	3025±1575
HFBG, mc2	951±3378	1506±1460	1869±7343	184.0±176.1
HFOT, mc2	2281±3846	513.5±3405	130±126.5	566.0±533.2
VLFBG, %	39.70±15.37*, ****	29.40±13.91*	30.10±16.60****	34.40±9.38
VLFOT, %	24.35±14.95	38.95±15.87	39.60±18.34	49.70±17.64
LFBG, %	26.10±11.88	33.50±14.37	33.80±14.52	30.10±14.45
LFOT, %	61.75±14.09	49.65±14.68	51.90±19.29	41.80±15.37
HFBG, %	28.80±15.62	34.00±12.75	35.80±16.52	29.20±13.46
HFOT, %	7.15 ±6,26	10.10±9.76	7.70±17.43	9.00±5.14
LFnuBG, %	46.75±22.03	46.80±15.88	48.20±19.20	55.40±20.27
LFnuOT, %	88.95±26.90	83.35±13.66	88.30±17.09	81.10±7.78
HFnuBG, %	47.35±19.90	53.20±15.88	51.80±20.61	44.60±20.27
HFnuOT, %	11.05±19.99	16.65±13.66	11.70±6.17	18.90±7.78
LF/HFBG	1.20±7.78	0.88±0.87	0.9±1.2	1.24±1.91
LF/HFOT	8.51±4.70	5.02±6.02	7.58±4.64	4.28±5.85

Note * - the difference Tukey's HSD test is reliable at $p < 0.05$ between the characteristics Group1. Group2. ** - it's reliable at $p < 0.05$ between Group2. Group3. *** - reliable at $p < 0.05$ between Group3. Group4. **** - reliable at $p < 0.05$ between Group1. Group4.

A physiological clinical explanation of the revealed patterns of the mobilization reaction can be given due to the representation of the human body as a bio-cybernetic model. The functional system of blood circulation regulation is a multi-circuit, hierarchical system with the dominant role of individual links according to the needs of the body in this model [1-3]. We share the opinion of a number of authors [4-6] that the two-circuit model of heart rate regulation R.M. Bayevsky (1978) [1, 2] is the simplest and most adequate model for clinical use. It is based on the idea of the existence of a central and autonomous levels (circuits) of regulation of cardiac activity with direct and feedback between them. The central circuit of the regulation of heart rhythm is a system of neurohumoral regulation of the physiological functions of the body by the central nervous system. The sinus node, vagus nerves and their nuclei in the medulla oblongata constitute an autonomous regulation loop (level of parasympathetic regulation). The frequency spectra of cardiac activity reflect the activity of regulation and can be recorded as electromagnetic phenomena by the HRV method. LF and HF represent the influence of the autonomous circuit. An increase in LF corresponds to activation and an increase in heart rate clinically. An increase in HF and a decrease in LF corresponds to inhibition and deceleration of the heart rate. VLF is the contribution of the central circuit to regulation and this indicator increases when central reserve mechanisms are included in the mobilization/adaptation process. Given this approach, we share the idea of [1, 3] and propose to classify the identified types of mobilization as follows:

1. Autonomous type – mobilization occurs due to the work of the autonomous circuit (this corresponded to type A and type C in our observation);

1.1. Mobilization occurs due to an increase in LF (it was type A in our observation): this is the most logical type of adaptation reaction based on the activation of the sympathoadrenal system;

1.2. mobilization occurs due to a decrease in all spectra (it was type C in our observation): this type is characteristic of well physically trained and overtrained individuals who have initially high energy spectral indicators of cardiac activity;

2. Reserve type – mobilization occurs using the central reserve mechanism: the transition to a vertical position is accompanied by an increase in VLF (this corresponded to type B and type D in our observation);

2.1. mobilization occurs due to an isolated increase in VLF (it was type B in our observation): this type can be considered as the initial variant of distress and impaired adaptation presumably;

2.2. mobilization with an increase in VLF and the entire spectrum: this type may turn out to be the most unfavorable since there is an initial decrease in all spectra, because it can be interpreted as an initially high level of randomness in the functioning of regulatory systems in the lying position and a decrease in randomness in the standing position. If an increase in all ranges occurs during rising, this indicates a simplification of the control mechanisms. That is, the body during mobilization is not able to maintain a high level of flexibility. This assumption is based on the idea that HRV is a reflection of the dynamic search for the optimal mode of work of the cardiovascular system of the body in the process of mobilization/adaptation.

The presented classification differs from the works [8, 9], which studied the type of the autonomic reaction to the orthostatic sample and distinguished only three types of reaction: autonomous, central, and autonomic-central variants.

Conclusions

1. Performing an orthostatic test in functionally healthy respondents causes a functional mobilization of the cardiovascular system in the form of one of four types A, B, C, D.

2. Types of mobilization/adaptation to an orthostatic test have clear mathematical laws of spectral dynamics of HRV and characteristic graphic visualization of dynamics.

3. The concept of a dual-circuit cybernetic model of regulation of cardiac activity R.M. Baevsky is most suitable for the clinical interpretation of the results.

4. The proposed classification is based on the cybernetic model of regulation of cardiac activity R.M. Baevsky also provides for dividing into two options, depending on the use of only the autonomous regulation circuit or, in addition, the inclusion of a central reserve circuit.

Prospects of further research are as follows. Using the proposed classification can give the doctor additional information about the functional state of the patient/respondent and become an additional criterion in assessing his valeological status, the efficacy of the treatment or training process. The use of a graphical diagnostic scheme as part of a dynamic analysis of spectral activity. It is planned to continue studies in this field and to study the functional types of mobilization in patients with NCDs.

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Реферати

ОЦІНКА ФУНКЦІОНАЛЬНИХ ТИПІВ
МОБІЛІЗАЦІЇ ОРГАНІЗМУ НА ПІДСТАВІ
ДИНАМІЧНОГО АНАЛІЗУ СПЕКТРАЛЬНИХ
ПОКАЗНИКІВ ВАРІАБЕЛЬНОСТІ РИТМУ
СЕРЦЯ ТА ЇХ КЛАСИФІКАЦІЯ

Невойт Г.В., Потяженко М.М., Минцер О.П.

Стаття присвячена вивченню електромагнітних феноменів серцевої діяльності людини і можливостям клінічного їх використання в практичній медицині для оцінки рівня здоров'я з метою профілактики НІЗ. У статті наведені результати динамічного аналізу спектральних показників серцевої діяльності при виконанні функціонально здоровими людьми ортостатичної проби. Автори вперше описали чотири функціональних типи мобілізації/адаптації, дали характеристику і інтерпретацію їм, запропонували варіант їх класифікації на основі кібернетичної моделі двохконтурної регуляції серцевої діяльності Р.М. Баєвського.

Ключові слова: варіабельність ритму серця, спектральний аналіз.

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ОЦЕНКА ФУНКЦИОНАЛЬНЫХ ТИПОВ
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ПОКАЗАТЕЛЕЙ ВАРИАБЕЛЬНОСТИ РИТМА
СЕРДЦА И ИХ КЛАССИФИКАЦИЯ

Невойт А.В., Потяженко М.М., Минцер О.П.

Статья посвящена изучению электромагнитных феноменов сердечной деятельности человека и возможностям клинического их использования в практической медицине для оценки уровня здоровья с целью профилактики НИЗ. В статье приведены результаты динамического анализа спектральных показателей сердечной деятельности при выполнении функционально здоровыми людьми ортостатического пробы. Авторы впервые описали четыре функциональных типа мобилизации/адаптации, дали характеристику и интерпретацию им, предложили вариант их классификации на основе кибернетической модели двухконтурной регуляции сердечной деятельности Р.М. Баевского.

Ключевые слова: вариабельность ритма сердца, спектральный анализ.

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UDC 616.831-002L.V. Pyra, R.V. Svistilnik, K.Yu. Romanchuk, O.O. Gordnichuk, D.G. Smolko
National Pyrogov Memorial Medical University, VinnytsyaCLINICAL AND EPIDEMIOLOGICAL CHARACTERISTICS OF ACUTE BACTERIAL
MENINGITIS IN ADULTS OF KHMELNYTSKYI REGION

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We analyzed 123 cases of acute bacterial meningitis, where 74 cases were in men and 49 cases - in women. 93.5% of cases began with fever, headache (86.2%), vomiting (69.9%), epileptic seizures (1.6%), altered level of consciousness (17.8%) and meningeal syndrome (80.2%). The etiological factor was established in 20.3% of patients. In 41.4% of patients neurological complications were observed. The terminal end was observed in 7.3% of patients. The main causative agent of acute bacterial meningitis was *S. pneumoniae* (56.0%), the second place was taken by *N. meningitidis* (36.0%), the third one was divided between *S. aureus* and *L. monocytogenes* (4.0%). Brain edema (90.2%), paresis of extremities (11.7%) and epileptic seizures (7.8%) were frequent complications. The prevalence of acute bacterial meningitis was 0.86 per 100 thousand population per year in the ratio between men and women of 1.5:1 and the total annual mortality of 0.06 per 100 thousand population.

Key words: bacterial meningitis, epidemiology, etiology, adults.

The study is a fragment of the research project "The course of infectious diseases depending on genetic, morphological and metabolic factors", state registration No. 0118U005454.

Acute bacterial meningitis (ABM) is one of the main causes of mortality from infectious diseases in the world [7]. Over the last few decades the incidence of ABM in children has decreased, but it leaves a significant burden of morbidity in adults with a mortality of about 30% [13].

The prevalence of ABM in average is 3 per 100 thousand population in the world and may vary depending on the age of patients, their sex and country [3].

In the United States in recent decades there have been some changes in the etiology and mortality of ABM with a tendency to decrease due to the introduction of combined vaccines from *Neisseria meningitidis* and *Streptococcus pneumoniae* and dexamethasone inclusion in the clinical protocols for the treatment [4].

The most common complication of the disease arises after meningitis caused by *S. pneumoniae* in comparison with other pathogens. Hearing disorder is one of the most common ABM complications. Other ABM complications include loss of limbs in the development of meningococcal sepsis, development of subdural empyema, hydrocephalus and epileptic seizures. The emergencies of neurocognitive impairment are frequent complications of ABM [13].

Three most common pathogens (*Haemophilus influenzae*-b, *N. meningitidis*, and *S. pneumoniae*) are responsible for more than 80% of ABM cases worldwide [4]. However, the epidemiology of ABM

pathogens can vary depending on the geographical location of the country, climatic conditions, age of patients and other factors. The study of such factors and the epidemiology of ABM especially the study of the etiological structure and its dynamics in time with the establishment of sensitivity to antibiotics is especially important for proper and timely control of morbidity [12].

The purpose of the work was to analyze the etiological structure, epidemiological features, socio-demographic profiles and the nature of the development of complications from the central nervous system in acute bacterial meningitis in adults of the Khmelnytsky region during 2007-2017.

Materials and methods. Prospective analysis of 123 cases of ABM was performed in adults, who were treated at Khmelnytskyi Infectious Diseases Hospital from 2007 to 2017. Of them, 74 patients were men and 49 were women.

The disease etiology was established by the simultaneous use of several methods for detecting the pathogen namely: bacterioscopy of the smear of cerebrospinal fluid with staining of the material on Gram, bacteriological sowing of blood and cerebrospinal fluid, detection of the genetic material of the pathogen in the liver by the PCR method.

The central nervous system complications were detected on the base of clinical picture of their development (paresis of extremities, epileptic seizures, etc.) and by additional research methods, mainly CT and MRI, both during the acute period of the disease and during the early convalescence. In the study we used an analytical method, and the processing of the results was carried out by means of determining the standard deviation of the arithmetic mean ($M \pm \sigma$), Student's criterion and constructing a 95% Confidence Interval (CI) for the mean difference.

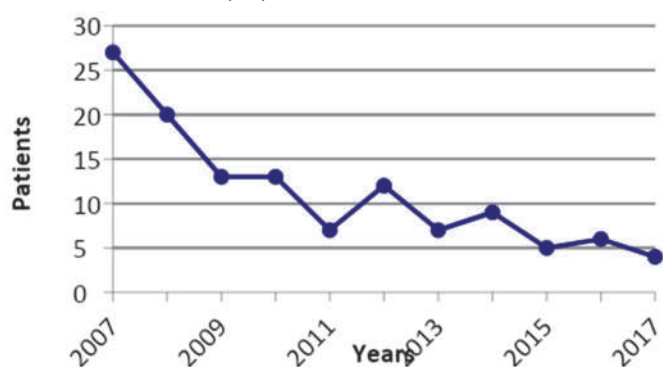


Fig. 1. The dynamics of ABM incidence rate in adults of Khmelnytskyi region during 2007-2017.

and a decrease in the number of adult population in the region) as well as the introduction of planned in Excise from Hib infection in children and diminishing its fate in the overall structure of the ABM.

ABM in adults arose in all seasons. In general the highest seasonal increase in the ABM incidence in adults was in August, and from October to December and made up 46.3% of the total annual incidence.

The lowest incidence was observed in January and February (5.7% and 4.9% respectively) as well as in June and July months (by 5.7%). Since August, there has been a sharp increase in the incidence

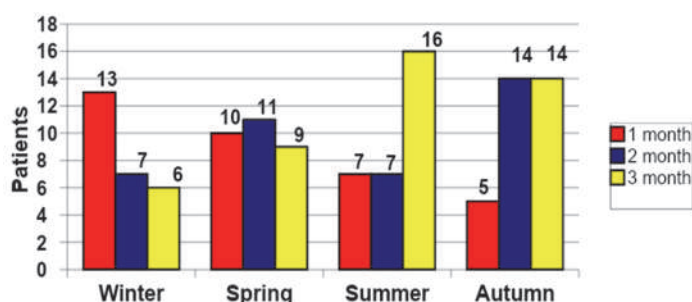


Fig. 2. Seasonal morbidity of ABM in adults in Khmelnytskyi Region from 2007 to 2017.

and from research depending on climatic, environmental and demographic factors.

An average age of adult patients with ABM was 41.2 ± 16.4 years. According to age categories, the ABM incidence was distributed as follows: in the young age from 18 to 39 years 62 (50.4%) patients were sick, with an average age of 40 to 59 years – 41 (33.3%) patients were sick, in the elderly from 60 to 79 years old – 19 (15.4%) patients were sick and older than 80 years – 1 (0.9%) patient was sick. Thus, the vast majority of ABM cases were found in young people aged 18-39 (50.4%) accounting for more than

Results of the study and their discussion. Analyzing the dynamics of the ABM morbidity in adults, it can be noted that the peak incidence occurred in 2007 and 2008. In the future, since 2009, the incidence has become mostly stable with small annual fluctuations and a gradual noticeable decrease to a minimum in 2017 as shown in the fig. 1.

First of all, the similar tendency of morbidity could be connected with social, demographic and climatic factors (especially by population migration and a decrease in the number of adult population in the region) as well as the introduction of planned in Excise from Hib infection in children and diminishing its fate in the overall structure of the ABM.

The increase in the ABM incidence in adults in the autumn period is most likely due to seasonal climatic changes and seasonal migration, increased respiratory diseases and increased number of contacts with other people.

Seasonal variation in the ABM incidence can vary from country to country

and from research depending on climatic, environmental and demographic factors.

half of adult patients. Probably this was due to the fact that this age category of the population is most socially active and primarily exposed to both environmental factors and social and demographic factors.

According to the data, the number of male patients with ABM was 74 (60.2%) people, while the female population was 49 (39.8%) patients which corresponded to the 1.5:1 of male to female ratio.

The etiological factor of ABM was established in 25 patients (20.3%) (fig. 3). The detection of bacterial agents in other countries and regions varies slightly and does not always reach even the average depending on the methods and availability of using the modern methods of verifying the pathogen, years of research and other factors.

In the Russian Federation the level of detection of ABM agents in the country was 37.1%, where the detection of meningococcal etiology was 41.8%, and not 32% of meningococcal etiology [10]. At the same time in Germany in the period from 2000-2009 the detectability of the pathogen was 83% [9].

Fig. 3 shows that *S. pneumoniae* is the main pathogen in adults among ABM which was detected in 15 (56.0%) cases. *N. meningitidis* took second place in ranked 9 (36.0%) patients. The third place was shared between *S. aureus* and *Listeria monocytogenes* which were detected in one case (4.0%).

It should be noted that in 6 (66.6%) cases the serogroup *N. meningitidis* was detected with the most frequent meningococcal serogroup B occurring in 3 (50.0%), in some cases serogroups A, C and W135 (by 16.6%).

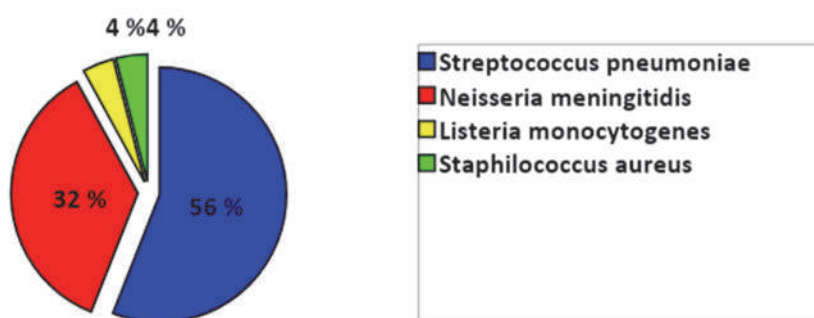


Fig. 3. Etiological structure of ABM in adults in Khmelnytskyi Region in 2007-2017.

The method of PCR cerebrospinal fluid was verified by the pathogen in 1 (4.0%) patient, bacterioscopy of the cerebrospinal fluid smear in 12 (48.0%) patients, by indirect hemagglutination test in 1 (4.0%) patient and by the method of bacteriological sowing of the material in a total of 20 (80.0%) patients. The

method of bacteriological cure of the cerebrospinal fluid was verified by the causative agent in 17 (85.0%) patients, sowing in 3 (15%) patients and in 1 (5.0%) of the patient the stem was sown simultaneously both in the blood and in the cerebrospinal fluid.

In some countries, the etiological structure of the ABM can differ from the usual one through the use of vaccines from the main pathogens of meningitis, as well as possibly geographic and climatic factors. However, in most countries *S. pneumoniae* is the key etiologic factor in adult ABM.

In Qatar, *Staphylococci* (20.1%) and *S. pneumoniae* (16.2%) were the most common pathogens of ABM. Among Gram-negative pathogens *Klebsiella pneumoniae* was detected in 10.2% of cases and *N. meningitidis* in 9.4% of cases [8].

In Germany, the study also revealed that *S. pneumoniae* was the main pathogen in adult's ABM cases and was detected in 50.5% of patients, *S. aureus* (10.5%) and *N. meningitidis* (9.4%) took the second and third places [9].

The prevalence of *S. pneumoniae* over other etiological factors of ABM in adults can be due to a significant percentage in the structure of bacterial meningitis secondary forms, which are mainly caused by *S. pneumoniae*. In our observation, the secondary nature of ABM was detected in 20 (16.2%) patients, where 7 (35%) patients were with the etiological factor of the disease which was *S. pneumoniae*. Among the main hearths of infection that led to the development of ABM were: purulent sinusitis (50%), purulent otitis (25%), mastoiditis (20%) and pneumonia (5%).

In the course of the studies, it was determined that *N. meningitidis* was susceptible to most antibacterial agents in all cases, namely: chloramphenicol, ampicillin, penicillin, ceftriaxone, meropenem, ofloxacin, amikacin, and gentamicin.

S. pneumoniae was almost always susceptible to levomycetin, ampicillin, penicillin, ceftriaxone, meropenem, ofloxacin, amikacin, vancomycin, ciprofloxacin, levofloxacin and rifampicin.

The clinical picture was characterized by severe courses in 120 (97.5%) patients and in 3 (2.5%) patients the course was moderately severe.

In 67 (54.5%) patients the disease was overwhelming as meningitis, whereas meningoencephalitis was diagnosed in 56 (45.5%) persons. In 13 (10.6%) people with meningococcal meningitis the disease was accompanied by the development of meningococemia.

The clinical manifestation of ABM is presented in table 1. In 93.5% of cases the disease began with fever, headache (86.2%), vomiting (76.4%), epileptic seizures (1.6%), altered level of consciousness (17.8%) Meningeal syndrome was detected in 80.2% of patients with neck stiffness.

Hyperesthesia was observed in 45.5% of patients and was manifested as a general excitability for sensory and tactile stimuli during examination and in the form of photophobia or phonophobia.

Table 1

Clinical manifestations of acute bacterial meningitis in adults

Clinical symptoms	Number of patients	%
Fever	115	93.5
Headache	106	86.2
Vomiting	86	69.9
Neck stiffness	98	79.6
Kernig's symptom	44	35.7
Symptom of Brudzinsky	17	13.8
Hyperesthesia	56	45.5
Stun	16	13.0
Sopor	6	4.9
Epileptic seizures	2	1.6
Focal Neurological Symptoms	29	23.6
Petechiae rash	13	10.6

Violation of consciousness was most often observed in the form of stunning (13.0%), less often in the form of a sopor (4.9%).

Focal neurological symptoms were presented in the form of the development of limb paresis in 1 (3.4%) patient, paresis of the cranial nerves in 7 (24.1%) patients, limb tone disorders, anisoreflexia and the presence of pathological reflexes in 21 (72.5%) patients. The debut of the disease in the form of petechial rash was exclusively observed in meningococcal meningitis with meningococemia.

In our observation, 51 (41.4%) patients experienced both acute and late neurological complications of the disease, much of them were severe (fig. 4).

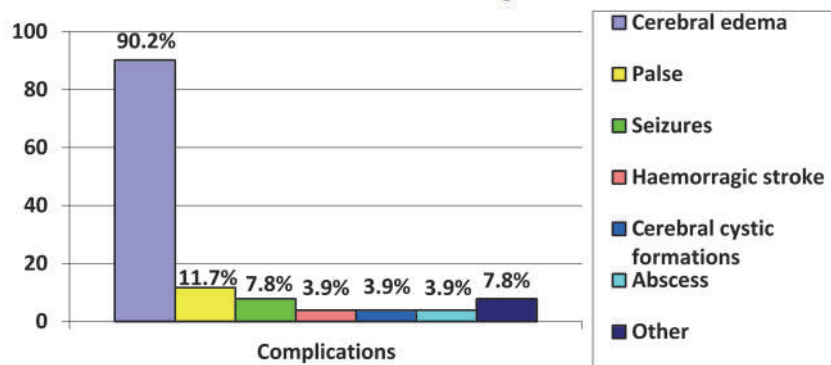


Fig. 4. General complications of ABM in adults from the central nervous system during the disease.

Of the severe and most frequent disorders that complicated the course of the disease, there were: cerebral edema (90.2%), paresis of extremities (11.7%), epileptic seizures (7.8%). There were also hemorrhagic strokes (3.9%), abscess formations (3.9%) and cerebral cystic formations (3.9%) detected.

In others, lesions (7.8%) in rare cases (1.9%) were subdural hygromas, ischemic stroke, lesions of cranial nerves and hydrocephalus. In 17 (33.3%) patients, several complications were observed simultaneously.

Among 51 patients who had severe complications, in 12 patients the pathogens were identified. In 10 (19.6%) patients *S. pneumoniae* was the pathogen and 2 (3.9%) patients had *N. meningitidis*.

The outcome of the disease with complete recovery was observed in 72 (58.5%) patients. In 18 (14.6%) patients, the consequences of the transmitted disease as asthenic syndrome were observed in 8 (44.4%) patients, autonomic disorders syndrome were observed in 2 (11.1%), epilepsy were observed in 1 (5.6%), hemiparesis were observed in 2 (11.1%), hydrocephalus were observed in 1 (5.6%) and paralytic obliquity were observed in 1 (5.6%) patient. In 3 (16.7%) patients were observed severe cognitive and behavioral disorders, which in the future required counseling and treatment by a psychiatrist.

Also during the study period 9 (7.3%) patients died, including 6 (66.6%) males and 3 (33.3%) females, which corresponded to a ratio of 2:1 with a total annual mortality of 0.06 per 100 thousand of adult population.

In all deceased patients the disease went through as meningoencephalitis. In virtually, all of the deceased the cause of death was the development of cerebral edema with insertion into a large occipital opening.

In 2 (22.2%) dead patients, the pathogens of *S. pneumoniae* were detected, as well as in the 3 (33.3%) cases the primary source of infection was detected.

According to our study, the mortality rate for ABM in adults was not higher in comparison with studies in other countries of the world, which can be explained by the improvement of timely diagnosis and care to patients with suspected ABM and improvement of the medical process using the treatment protocols and worldwide recommendations for this pathology. At the same time, the percentage of mortality from the ABM is slightly different from each other according to various studies and may depend on the prevalence of the structure of the disease of an etiological factor, age division of patients, treatment approach and the country.

Thus, in the German observation for 10 years of studying ABM in adult's, total mortality was 15% [9]. In the Iranian study the mortality from ABM was 8.3% [6].

The development of similar clinical symptoms characteristic of ABM in different ratios was also noted in studies by other authors.

According to Wei-An Lai et al, (2011), with ABM in adults fever was detected in 86% of cases, disturbance in consciousness was detected in 62%, epileptic seizures in 30%, leukocytosis in 53%, and an infectious and toxic shock in 11% of patients [11].

In the study of F.Y. Khan et al, (2017) fever was observed in 94.0% of adult patients, altered level of consciousness in 47.0%, headache in 36.6%, vomiting in 29.9%, meningeal symptoms in 26.5%, and epileptic seizures in 19.7% of patients [8].

Sometimes divergent clinical data of the studies from different countries could be explained by different age structures of patients (prevalence of elderly patients or conversely), presence of concomitant pathology, etiological factor of ABM and other reasons which determines the importance and necessity of research of clinical symptoms in separate regions and countries.

The highest level of neutrophil cytositis was observed in meningitis caused by *N. meningitidis* which was 5815.8 ± 4436.8 cl/mm³ and significantly exceeded the cytositis level in meningitis caused by *S. pneumoniae*, at which it reached 2659.5 ± 2004.5 cl/mm³ ($p < 0.03$) (95% CI, 306.5 to 6006.0 cl/mm³).

The high level of cytositis in meningitis-induced *N. meningitidis* can be explained by a significant inflammatory response of the organism in response to the release of endotoxin from bacteria, the main factor of the occurrence of infectious and toxic shock, which is released only when bacteria break down, which is especially characteristic of meningococcus and other Gram-negative bacteria. The toxic effect of endotoxin causes the stimulation of cells of the immune system, which leads to the release of a large number of inflammatory mediators and cytokines with the development of severe inflammatory response.

On the contrary, the level of cerebrospinal fluid protein was the highest with meningitis caused by *S. pneumoniae* and composed 1.77 ± 0.60 g/l in comparison with meningitis-induced *N. meningitidis*, where its level reached 1.14 ± 0.53 g/l ($p < 0.02$) (95% CI, 0.09 to 1.16 g/l). Such a difference can be explained by the fact that the meningitis caused by *S. pneumoniae* has a more severe course and is much more likely to cause the brain lesions with the development of neurological complications and the tendency to abscess due to the influence of exotoxin pneumolysin [2].

Neurological complications that arise with ABM can increase the likelihood of a fatal end and lead to patients' disability [7]. Cerebral vascular complications with ABM can include the development of ischemic and hemorrhagic stroke [7]. The development of ischemic stroke with ABM promotes the vasculitis of cerebral vessels, vascular spasm and their thrombosis. [9]. According to J. Bodilsen et al. (2014), an ischemic stroke occurs in 8-25% of patients with ABM [1]. Hemorrhagic stroke is a less common complication in ABM and occurs in 3-9% of patients, mainly with meningitis-induced *S. pneumoniae* [5]. Inflammation and pressure increased in the subarachnoid space can cause cranial neuropathy. Damage of the brain structures contributes to the development of psycho-neurological disorders and cognitive disorders [7]. In the study of Heydari B. et al. (2016) the development of neurological complications in the form of cranial nerves paralysis arose in 11.1% of cases, hearing impairment - in 5.5% of cases and hemiparesis - in 2.8% of cases of 36 patients. In total, complications from the central nervous system appeared in 7 patients (19.4%) [6]. Early detection of such complications and their treatment may reduce the ABM mortality and its consequences [7].

Taking into account epidemiological data, in the course of the study the prevalence of ABM among adults in the Khmelnytskyi region was determined. In total it was 0.86 per 100 thousand adult population per year. It can be noted that the obtained indicator is close to the general indicator for the European population, which, according to EFNS data is 1-3 per 100 thousand populations.

Conclusion

The annual dynamics of ABM morbidity in adults is predominantly stable and occurs during all seasons with slight annual fluctuations and a marked peak in 2007-2008 and a gradual decrease to a minimum in 2017. The average age of adult patients with ABM was 41.2±16.4 years. Mostly young people aged 18-39 years (50.4%) are diagnosed with ABM. The disease is more common in men than in women in the ratio of 1.5:1. The etiological factor of ABM was established in 25 (20.3%) patients. *S. pneumoniae* are the main pathogens among adults with ABM and were detected in 14 (56.0%) cases. *N. meningitidis* took second place and was detected in 8 (32.0%) patients. The third place was shared between *S. aureus* and *Listeria monocytogenes* which was detected in 1 case (4.0%).

In our observation, 51 (41.4%) patients had neurological complications of the disease. The most frequent complications were cerebral edema (90.2%), limb paresis (11.7%), epileptic seizures (7.8%). The most severe cerebral complications occurred with meningitis caused by *S. pneumoniae*. The outcome of the disease with complete recovery was observed in 72 (58.5%) patients and in 18 (14.6%) patients, the consequences of the disease were observed.

During the study, 9 (7.3%) patients died, which corresponded to an annual mortality of 0.06 per 100 thousand adult population. During the study, the prevalence of ABM among adults in the Khmelnitskyi region was determined. In total it was 0.86 per 100 thousand per year. It should also be noted that the incidence of ABM in adults tends to decrease. If by 2012 the prevalence of ABM among the adult population was 1.2 per 100 thousand, then from 2012 to 2017 it already amounted to 0.5 per 100 thousand. A similar trend can be explained by a decrease in the population of the region in recent years, as well as the introduction of planned vaccination against Hib infection in childhood and reducing its share in the structure of ABM in adults.

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Реферати

**КЛІНІКО-ЕПІДЕМІОЛОГІЧНА
ХАРАКТЕРИСТИКА ГОСТРИХ
БАКТЕРІАЛЬНИХ МЕНІНГІТІВ
У ДОРОСЛИХ ХМЕЛЬНИЦЬКОЇ ОБЛАСТІ**

**Пипа Л.В., Свістільник Р.В., Романчук К.Ю.,
Гордійчук О.О., Смолко Д.Г.**

Проведений аналіз 123 випадків гострих бактеріальних менінгітів, 74 з яких у чоловіків і у 49 жінок. У 93.5% випадків захворювання розпочиналось з

**КЛИНИКО-ЭПИДЕМИОЛОГИЧЕСКАЯ
ХАРАКТЕРИСТИКА ОСТРОГО
БАКТЕРИАЛЬНОГО МЕНИНГИТА
У ВЗРОСЛЫХ ХМЕЛЬНИЦКОЙ**

**Пипа Л.В., Свистильник Р.В., Романчук К.Ю.,
Гордийчук О.А., Смолко Д.Г.**

Проведен анализ 123 случаев острого бактериального менингита, 74 из которых у мужчин и 49 у женщин. В 93.5% случаев заболевание начиналось с

лихоманки, головного болю (86.2%), блювоти (69.9%), судом (1.6%), порушення свідомості (17.8%) і менингеального синдрому (80.2%). Етіологічний чинник був встановлений у 20.3% пацієнтів. У 41.4% пацієнтів спостерігались неврологічні ускладнення. Летальний наслідок спостерігався у 7.3% пацієнтів. Головним збудником гострих бактеріальних менингітів виявився *S. pneumoniae* – 56.0%, друге місце посіла *N. meningitidis* – 36.0%, третє поділили між собою *S. aureus* і *L. monocytogenes* (по 4.0% випадків). Частими ускладненнями виявлялися набряк мозку (90.2%), парези кінцівок (11.7%) і епілептичні припадки (7.8%). Розповсюдженість гострих бактеріальних менингітів склала 0.86 на 100 тис населення в рік в співвідношенні між чоловіками і жінками 1.5:1 та загальною річною смертністю 0.06 на 100 тис населення.

Ключові слова: бактеріальний менингіт, епідеміологія, етіологія, дорослі.

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лихорадки, головної болю (86.2%), рвоти (69.9%), судорог (1.6%), порушення свідомості (17.8%) і менингеального синдрому (80.2%). Етіологічний фактор був встановлений у 20.3% пацієнтів. У 41.4% пацієнтів спостерігались неврологічні ускладнення. Летальний наслідок спостерігався у 7.3% пацієнтів. Головним збудником гострого бактеріального менингіту виявився *S. pneumoniae* – 56.0%, друге місце посіла *N. meningitidis* – 36.0%, третє поділили між собою *S. aureus* і *L. monocytogenes* (по 4.0% випадків). Частими ускладненнями були отек мозку (90.2%), парези кінцівок (11.7%) і епілептичні припадки (7.8%). Розповсюдженість гострого бактеріального менингіту склала 0.86 на 100 тис населення в рік в співвідношенні між чоловіками і жінками 1,5:1 та загальною річною смертністю 0.06 на 100 тис населення.

Ключевые слова: бактериальный менингит, эпидемиология, этиология, взрослые.

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MORPHOLOGICAL JUSTIFICATION OF THE STEPWISE DOSED BALLOON ANGIOPLASTY APPLICATION COMPARED TO STANDARD METHODS IN PATIENTS WITH DIABETIC FOOT

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The main reason for the development of lower extremities gangrene in diabetes mellitus is impairment of their blood supply. In recent decades, endovascular surgical treatment has played a significant role in restoring blood supply. However, the issues of complications that arise after such interventions, the reasons for their occurrence, what morphological changes occur in the atherosclerotic artery wall during their implementation remain understudied. Therefore, experimental research to study the local changes that occur in the arteries of the lower extremities during balloon angioplasty, especially in patients with ischemic diabetic foot, is an urgent problem of modern interventional surgery. The purpose of the study was to provide a morphological justification for the use of stepwise dosed balloon angioplasty using balloons of different diameters and lengths compared to standard methods in patients with ischemic form of diabetic foot syndrome. An experimental study was performed on 20 lower limbs that were amputated at the hip level for foot gangrene in patients with ischemic form of diabetic foot syndrome. The first group included 5 (25.0%) lower extremities, in which immediately after the surgery, sections of the tibial arteries with stenosis of more than 75% were sampled. Group II included 5 (25.0%) amputated lower extremities, which immediately after the surgery were performed a typical balloon angioplasty. Group III included 10 (50%) amputated lower extremities, in which the method of stepwise dosed balloon angioplasty was tested. According to the results of experimental studies, it was found that mainly when performing staged dosed angioplasty according to the proposed method, the inner elastic membrane of the artery was clearly pronounced, had insignificant areas of fragmentation. At the same time, the outer elastic membrane was quite well pronounced throughout the whole length, had insignificant areas of fragmentation and they were much less in number than in those cases when angioplasty was performed according to standard methods. Moreover, in the outer membrane, where vasa vasorum and vascular nerves were located, they remained almost unchanged. Reducing the number and sizes of arterial membranes' dissection, their fragmentation when performing staged dosed balloon angioplasty by the proposed method using balloons of different diameters and lengths permits to recommend it in practice to reduce the incidence of thrombotic complications in the early postoperative period.

Key words: arteries, atherosclerosis, arterial occlusion, diabetes mellitus, diabetic foot, balloon angioplasty.

The work is a fragment of the research project "Development of modern scientifically based principles of stratification, monitoring and prognostication of surgical diseases and injuries course", state registration No.0120U101176.

Over the past 10-15 years, the incidence of diabetes mellitus (DM) in Ukraine has almost doubled to more than 1.5 million people. The syndrome of diabetic foot (DFS) occurs in every fourth patient and ends in almost half with high amputation of one or both lower extremities [3]. Two thirds of patients die from gangrene of the lower extremities, and its development in patients with diabetes is observed many-fold more frequently than in the general population. [6, 7].

The main reason for the development of the lower extremities gangrene in diabetes mellitus is an impairment of their blood supply [11, 12]. The main method of restoring blood flow in this disease is surgical revascularization, but this method can be used to a limited extent in patients with virtually no distal blood flow and with the presence of severe comorbidities. In addition, there is currently no reliable

assessment criterion that permits to select patients for surgical revascularization [1]. The situation is further complicated by the fact that against the background of diabetes mellitus, the infragenicular arteries are most often affected [3, 7, 12].

At the same time, the problems of complications after endovascular intervention for chronic ischemia of the lower extremities and technical difficulties during their implementation are insufficiently studied and clarified in the domestic and world literature [5]. There is almost no literature on morphological changes that occur in atherosclerotic artery wall during balloon angioplasty in patients with diabetes mellitus. Depending on these data, it is possible to predict the development of some complications that occur in the early postoperative period after endovascular interventions [10]. Therefore, experimental study of local changes that occur in peripheral arteries during balloon angioplasty, particularly in patients with ischemic form of DFS, is an urgent problem of modern interventional surgery, [9].

The purpose of the study was to provide a morphological justification for the use of stepwise dosed balloon angioplasty using balloons of different diameters and lengths compared to the standard method in patients with ischemic form of diabetic foot syndrome.

Materials and methods. The study was performed on 20 lower limbs that were amputated at the level of the thigh for foot gangrene in patients with ischemic DFS. This experimental study was approved by the Commission on Ethics and Bioethics of the Ukrainian Medical Stomatological Academy at the Ministry of Health of Ukraine.

Amputated limbs for the experiment were divided into 3 groups. Group 1 consisted of 5 lower extremities, in which immediately after the operation, sections of the infragenicular arteries were taken at the site of stenosis (75% or more). Group 2 also included 5 lower extremities, on which immediately after the operation an experimental typical one-stage conventional angioplasty under pressure from 8 to 22 atm was performed with antegrade (through the femoral artery) insertion of a Pacific Plus OTW balloon into the posterior or anterior infragenicular arteries. Group 3 included 10 (50%) amputated lower extremities, on which we tested our proposed method of stepwise dosed balloon angioplasty.

The method of stepwise dosed balloon angioplasty lied in the fact that in the lumen of one of the infragenicular arteries a conductor was inserted behind the affected area, through which a balloon catheter for angioplasty was inserted and there was a gradual increase in pressure with exposure for 5 minutes at 3/4 of the nominal pressure with gradual pressure increase by 1 atm for 1 minute, and exposure for 5 minutes when reaching the required diameter of the balloon catheter. This study was performed on areas of the posterior or anterior great infragenicular arteries up to 10 cm long, with balloons corresponding to the diameter of the selected vessel and ranging from 2 to 3.5 mm and the length of 2 to 4 cm. Raising the pressure in the balloon causes the gradual influence on the arterial wall, reduces its trauma and helps to preserve the integrity of the arterial intima (performed under the control of arteriography).

General histological study methods were applied.

Results of the study and their discussion. Morphological changes in the walls of the infragenicular arteries of group 1 in atherosclerosis were evidence of its initial stages. In the cross sections of the vessels, the intima at the site of atherosclerotic plaque had the form of an intermittent lining, due to the partial destruction of endothelial cells. Undamaged endothelial cells had unevenly spaced hyperchromic nuclei that protrude into the vascular lumen. The walls were segmentally thickened with the formation of plaques, which were based on actively proliferating fibroblasts and cells of smooth myocytes of the subendothelial layer. The nuclei of the latter in these places were arranged in several layers, perpendicular to the inner surface of the vessel. The fibrous structures of the plaque in places had a loose appearance, which was an evidence of mucoid edema. The middle membrane of the arteries under the plaque was thin. The outer elastic membrane was without visible changes. The loose fibrous connective tissue of the adventitia contained unevenly full-blooded blood vessels located in small groups.

Assessing the results of histological examination of the infragenicular arteries of group 2 (after angioplasty according to the standard method, in which the selected diameter cylinders were installed in the site of atherosclerotic lesions of the vessel with the simultaneous achievement of the required balloon diameter) showed that the inner elastic membrane was loosened and exfoliated. In some areas, exfoliation of the intima and inner elastic membrane with a layer of smooth myocytes of the middle membrane was observed in the places of ballooning (fig. 1A).

In the media membrane, on the border with adventitia, small hemorrhages were detected in the intercellular space. The outer elastic membrane was not clearly visualized, but areas of collagen and elastic fibers fragmentation were also observed in it (fig. 1B).

The outer membrane of the artery was represented by loose fibrous connective tissue, in which perivascular cell infiltration was determined.

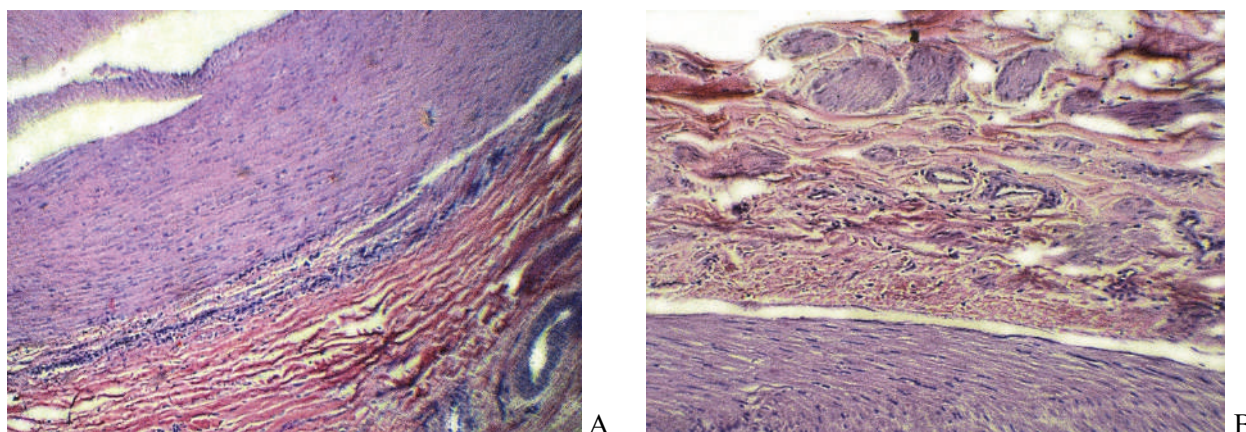


Fig. 1. The wall of the anterior great infragenicular artery after balloon angioplasty by standard methods: A - exfoliation of the intima with a layer of smooth myocytes of the media; B - adventitial exfoliation with hemorrhage. Hematoxylin and eosin staining. Magnification: Oc: 10; Ob.10

In group 3, histological examination of the anterior infragenicular artery wall, when performing balloon angioplasty by our proposed stepwise, dosing method, showed that the inner membrane consisted of endothelial cells, which had a polygonal shape and were located on the inner elastic membrane (fig. 2A).

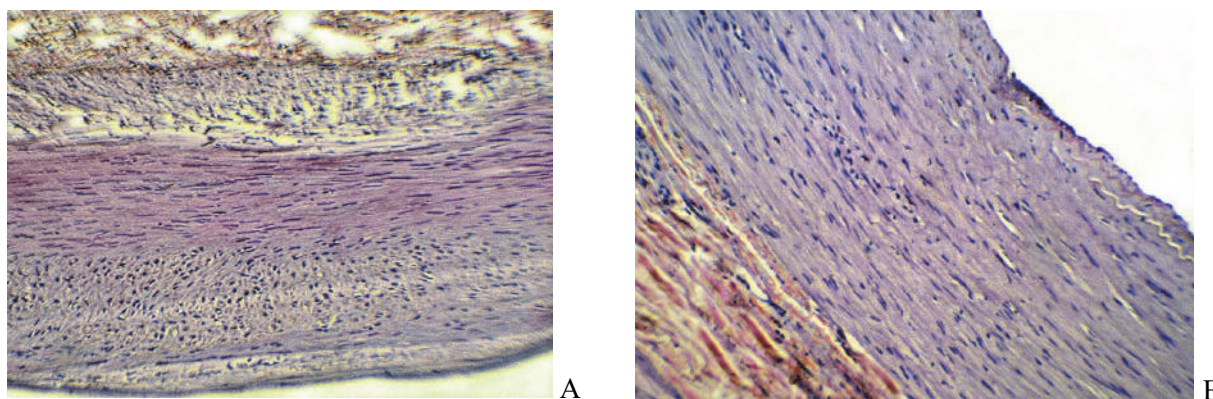


Fig. 2. The wall of the anterior infragenicular artery during balloon angioplasty by stepwise, dosed achievement of nominal pressure in the balloon: A - preservation of intima, media and adventitia; B - slight stratification in the media and outer membranes. Hematoxylin and eosin staining. Magnification: Oc: 10; Ob.10.

Endothelial cells were stretched along the vessel, the subendothelial layer consisted of thin elastic and collagen fibers, which were also located along the vessel. Single smooth myocytes and low specialized connective tissue cells were located between the fibers. The inner elastic membrane was clearly defined, had insignificant areas of fragmentation, almost a continuous contour. In the specimens there were single areas of intima and internal elastic membrane exfoliation (fig. 2B).

The media membrane consisted of smooth myocytes, between which there were single collagen and elastic fibers, making a framework for them (fig. 2A, B).

Comparing the architectonics of the media membrane to group 2 (balloon angioplasty, which was performed according to the standard method), it was found that the stepwise, dosed method of balloon angioplasty showed a lower frequency of these stratifications and their insignificant size.

The outer elastic membrane was quite well pronounced throughout, but had insignificant areas of fragmentation and at the same time, they were less numerous than in cases where angioplasty was performed according to standard methods.

The outer membrane was represented by loose fibrous connective tissue in which the vasa vasorum and the nerves of the vessels were localized, which remained almost unchanged in the analysis of histological specimens.

Due to the rapid development of endovascular surgery, it has become possible to use endovascular revascularization methods for distal lesions. This largely applies to balloon angioplasty and stenting of the infragenicular arteries. Recently, the use of balloon angioplasty is proposed to be considered in patients with critical lower extremity ischemia as a first-line intervention [12].

Prospects for the use of balloon angioplasty were determined by the following factors: achieving adequate results, reducing the number of complications, the possibility of repeated interventions, low mortality. All this opened up great opportunities in the application of this method in the treatment of critical ischemia of the lower extremities. Endovascular treatment methods had a relatively low risk of

complications with a high level of success [12], therefore some researchers predict the technical success of endovascular treatment in impaired patency of the lower extremities arteries at 91% [4].

Therefore, according to experimental research on histological study of changes that occurred in the atherosclerotic wall of the infragenicular arteries and during balloon dilatation in various ways, it was established that mainly when performing stepwise dosed angioplasty according to the proposed method, the internal elastic artery membrane was sufficiently pronounced, had insignificant fragmentations with an almost continuous contour. At the same time, the outer elastic membrane was quite well pronounced throughout, had insignificant areas of fragmentation and they were much smaller than in those cases when angioplasty was performed according to standard methods. Moreover, the outer membrane was represented by loose fibrous connective tissue, where there were vasa vasorum and vascular nerves, which remained almost unchanged. The obtained data do not contradict those previously published [2].

Conclusion

Reducing the number and size of arterial membranes exfoliations, their fragmentation when performing stepwise dosed balloon angioplasty according to the proposed method using balloons of different diameters and lengths, permits to recommend its use in practice to reduce the incidence of thrombotic complications in the early postoperative period.

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Реферати

**МОРФОЛОГІЧНЕ ОБҐРУНТУВАННЯ
ЗАСТОСУВАННЯ ПОЕТАПНОЇ ДОЗОВАНОЇ
БАЛОННОЇ АНГІОПЛАСТИКИ У ПОРІВНЯННІ
ЗІ СТАНДАРТНОЮ МЕТОДИКОЮ У ХВОРИХ
З ШЕМИЧНОЮ ФОРМОЮ СИНДРОМУ
ДІАБЕТИЧНОЇ СТОПИ**

Пузырьов Г.С., Ляховський В.І., Шепітько В.І.,
Сидоренко А.В.

В останні десятиліття значне місце у відновленні кровообігу відіграють ендovasкулярні методи хірургічного лікування. Однак, залишаються недостатньо вивченими питання ускладнень, які виникають після

**МОРФОЛОГИЧЕСКОЕ ОБОСНОВАНИЕ
ПРИМЕНЕНИЯ ПОЭТАПНОЙ ДОЗИРОВАННОЙ
БАЛОННОЙ АНГИОПЛАСТИКИ В СРАВНЕНИИ
СО СТАНДАРТНОЙ МЕТОДИКОЙ У БОЛЬНЫХ
С ИШЕМИЧЕСКОЙ ФОРМОЙ СИНДРОМА
ДИАБЕТИЧЕСКОЙ СТОПЫ**

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В последние десятилетия значительное место в восстановлении кровоснабжения играют эндоваскулярные методы хирургического лечения. Однако, остаются недостаточно изученными вопросы осложнений, которые

проведення таких втручань, причини їх виникнення, які морфологічні зміни відбуваються у атеросклеротично змінених стінці артерії при їх проведенні. Метою дослідження було дати морфологічне обґрунтування застосування поетапної дозованої балонної ангиопластики з використанням балонів різного діаметру і довжини у порівнянні з стандартною методикою у хворих з ішемічною формою синдрому діабетичної стопи. Згідно отриманих результатів експериментальних досліджень встановлено, що в основному при виконанні поетапної дозованої ангиопластики за запропонованим способом внутрішня еластична перетинка артерії чітко виражена, має не значні ділянки фрагментації. При цьому, зовнішня еластична перетинка виражена достатньо добре на всьому протязі, має не значні ділянки фрагментації і їх було значно менше чим у тих випадках коли виконувалась ангиопластика за стандартною методикою. Причому, у зовнішній оболонці, де знаходилися судини судин та нерви судин, вони збереглися майже без змін. Зменшення кількості і величини розшарувань оболонок артерії, їх фрагментації при виконанні поетапної дозованої балонної ангиопластики за запропонованим способом з використанням балонів різного діаметру і довжини дозволяє рекомендувати її використання у практичній діяльності для зменшення частоти виникнення тромботичних ускладнень у ранньому післяопераційному періоді.

Ключові слова: діабетична стопа, балонна ангиопластика

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возникают после проведения таких вмешательств, причины их возникновения, какие морфологические изменения происходят в атеросклеротически измененной стенке артерии при их проведении. Целью исследования было изучить в эксперименте морфологические изменения, которые происходят в стенке берцовых артерий при проведении баллонной ангиопластики у больных с ишемической формой синдрома диабетической стопы. Согласно полученным результатам экспериментальных исследований установлено, что в основном при выполнении поэтапной дозированной ангиопластики по предложенному способу внутренняя эластичная перепонка артерии четко выражена, имеет не значительные участки фрагментации. При этом, внешняя эластичная перепонка выражена достаточно хорошо на всем протяжении, имеет не значительные участки фрагментации и их количество было значительно меньше чем в тех случаях, когда выполнялась ангиопластика по стандартной методике. Причем, во внешней оболочке, где находились сосуды сосудов и нервы сосудов, они сохранились почти без изменений. Уменьшение количества и величины расслоений оболочек артерии, их фрагментации при выполнении поэтапной дозированной баллонной ангиопластики по предложенному способу с использованием баллонов различного диаметра и длины позволяет рекомендовать ее применять в практической деятельности для уменьшения частоты тромботических осложнений в раннем послеоперационном периоде.

Ключевые слова: диабетическая стопа, балонная ангиопластика

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NOVEL NON-INVASIVE SEVERITY MARKERS IN IDIOPATHIC PULMONARY FIBROSIS

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Idiopathic pulmonary fibrosis is a severe, steadily progressive disease. Lack of specific signs and presence of individual variations in the course of the disease indicate the need to find additional non-invasive markers for diagnosis, estimation of the disease severity and monitoring of treatment effectiveness. Therefore, this study aimed to determine activities of gelatinase A and gelatinase B activities, as well as progelatinase B/lipocalin complex in patients with moderate and severe IPF. It was found that increased gelatinase A and gelatinase B activities correlated with the disease progression. Increased activities of progelatinase B and its active form at different stages of the disease can be used as markers of the severity of the fibrotic process, while gelatinase A activity can indicate its stage. Changes in the progelatinase B/lipocalin complex activity reflect clinical signs and symptoms during the idiopathic pulmonary fibrosis course and are associated with the severity of the disease.

Keywords: idiopathic pulmonary fibrosis, gelatinases A and B, progelatinase B/lipocalin complex.

The work is a fragment of the research projects "Improving of diagnosis, comprehensive prevention and treatment of respiratory and comorbid diseases in industrial workers and residents of the industrial area", state registration No. 0117U004787 and "Pharmacological approaches on prevention of respiratory failure development in patients with a chronic obstructive pulmonary disease (COPD) in combination with cardiovascular diseases", state registration No. 0115U002017.

Idiopathic pulmonary fibrosis (IPF) is a severe and potentially fatal disease, which is defined by a radiological and histopathological pattern of interstitial pneumonia. The triggering mechanisms of this disease remain unclear [12]. The diagnosis of IPF is based on a set of clinical signs, lung biopsy data, and a typical high-resolution computed tomography pattern [11], provided that other diseases that cause pulmonary fibrosis are excluded [13]. IPF predominantly affects elderly patients. Among the adult population, IPF is usually diagnosed in patients older than 55 years. The average survival rate is 3-5 years and it directly depends on the patient's age at the time of diagnosis: in patients diagnosed between 66 and 69 years of age the survival was almost 8 years compared with 4.5 years in patients diagnosed at the age of 75-79 years and only 2.5 years in patients over 80 years of age [10].

Clinical signs of IPF are not sufficiently specific and coincide with those of other diseases of the interstitial lung disease group [1]. Currently, the high-resolution computed tomography has been

considered the gold standard for diagnosis of IPF, and pulmonary function parameters are usually measured for patient monitoring. However, given the variable nature of the disease course, it is important to find some sensitive, non-invasive, and reliable markers of IPF that could be used as an aid in IPF diagnosis, estimation of the severity, and monitoring of the treatment effectiveness. One of the promising areas of research includes investigation of proteolytic processes, the activity of which changes in IPF.

An important role in the IPF pathogenesis is played by a cumulative action of multiple processes that trigger the pathogenic cascade leading to abnormal activation of epithelial cells [9]. This results in the secretion of multiple mediators that promote an increase in the fibroblast population and contribute to the uncontrolled remodelling of the extracellular matrix (ECM), which, in turn, leads to an excessive accumulation of proteins, mainly collagen, and angiogenesis. In the early stages of IPF, apoptosis of type 1 pneumocytes results in proliferation of type 2 pneumocytes [8]. The latter, together with alveolar macrophages, neutrophils, lymphocytes and fibroblasts, produce profibrotic cytokines such as transforming growth factor β 1, fibroblast growth factor, insulin-like and epidermal growth factors, which induce differentiation of myofibroblasts (contractile fibroblasts) and formation of fibroblast clusters serving as new loci of deposition of extracellular matrix proteins [14]. In addition, all of these cells synthesize a significant number of different mediators and matrix degrading enzymes that, under physiological conditions, play a leading role in maintaining the extracellular matrix homeostasis. Such enzymes include calcium-dependent zinc gelatinases A and B (matrix metalloproteinases MMP2 and MMP9, respectively), which control metabolic processes via their effects on functional activities of other enzymes and growth factors, and degrade most extracellular matrix proteins, including basement membrane proteins. These proteases promote adhesion and transendothelial migration of fibroblasts/myofibroblasts, thereby altering the lung microenvironment [3, 9].

Neutrophils play an extremely important role. Specific granules of neutrophils contain a small glycosylated protein from the lipocalin family, also known as neutrophil gelatinase-associated lipocalin (NGAL). NGAL has a molecular mass of approx. 25kDa. This protein is released from neutrophil granules in monomeric, homodimeric (45-50 kDa), homotrimeric (approx. 70 kDa) and heterodimeric forms (lipocalin monomer disulphide-linked to proMMP9; 130-135 kDa) [6]. The proMMP9/NGAL complex was found to play an important role in acute renal failure, cardiovascular disease, carcinogenesis and metastatic processes, as well as in chronic obstructive pulmonary disease [4, 5]. However, there is almost no information about patterns of changes in proteolytic activities of gelatinases A and B and the proMMP9/NGAL complex in IPF.

The purpose of the study was to study the activities of matrix metalloproteinases 2 and 9 and the proMMP9/NGAL complex in blood plasma of patients with idiopathic pulmonary fibrosis vary depending on the severity of the disease.

Materials and methods. The total of 25 patients with IPF including 19 women and 6 men between 40 and 77 years of age participated in this study. The patients were diagnosed with IPF 1 month to 4 years prior to their inclusion in the study (mean disease duration was 5 (3-12) months). IPF was diagnosed based on the clinical and radiological criteria recommended by the Association of Tuberculosis Specialists and Pulmonologists of Ukraine [3] and ATS/ERS/JRS/ALAT diagnostic and treatment criteria [16]. After the Informed Consent to voluntarily participate in the study had been signed, the patients were enrolled in the study, provided they met the following criteria: age between 40 and 80 years, verified IPF diagnosis, no history of any of the following: other lung pathology, bronchial asthma, HIV/AIDS, hepatitis B or C.

All patients stayed in a specialized hospital during the study. At the time of admission to the hospital (day 1), and then after one month and after one year from the treatment beginning, the following tests were performed for all patients: full blood count and urinalysis, blood biochemistry, blood coagulation test, and pulse oximetry to determine the level of oxygen saturation, in addition, dyspnea was assessed by the modified Medical Research Council (mMRC) scale, and body mass index (BMI) was calculated. The standard treatment regimen was in compliance with domestic and international standards [3, 11, 16].

Analysis of clinical data was based on evaluation of symptoms using the modified Wood-Downes scoring system (table 1), oxygen saturation level and the best of the three values measured during spirometry (forced expiratory volume in 1 second (FEV1), peak expiratory flow rate (PEFR)), and based on these data patients were divided into 2 groups [16].

Group 1 included 12 patients with moderately severe IPF, Group 2 included 13 patients with IPF whose condition was evaluated as severe. The Control included 15 age-matched healthy volunteers.

The activities of MMP2/9 and the proMMP9/NGAL complex were evaluated using gelatin zymography. After vertical gel electrophoresis of blood plasma samples in 7.5% polyacrylamide gel containing 0.1% sodium dodecyl sulphate and 1% gelatin substrate, the gels were washed four times for 15

min in 2.5% Triton X-100. Next, the gels were incubated at 37°C for 24 hours in buffer containing 25 mmol/L Tris-HCl, 5 mmol/L CaCl₂, 0.9% NaCl, 0.05% NaN₃ (pH 7,5). At the end of incubation, the gels were stained with 1% Coomassie Brilliant Blue G-250 in 40% methanol containing 10% acetic acid. MMPs appeared as transparent bands against the blue background.

Table 1

Modified Wood-Downes scale

	1	2	3
Respiratory rate	Normal or exceeding the age-appropriate reference value by up to 30%	Exceeds the age-appropriate reference value by 30-50%	Exceeds the age-appropriate reference value by >50%
Oxygen saturation	>95%	90-95%	<90%
Auscultatory findings	Moderate wheezing at the end of exhalation	"Mosaic" breathing pattern, significant wheezing during exhalation	Weakened breathing, significant wheezing during prolonged exhalation, crackles
Use of accessory muscles for respiration	No	Intercostal and subcostal muscles take part in respiration (mild or moderate degree)	Involvement of intercostal, subcostal, suprachoroidal muscles (significant degree), paradoxical breathing
State of consciousness	Not impaired	Moderately exalted	Exalted consciousness
PEFR (% of the reference value)	70-90%	50-70%	<50%

Colored markers for electrophoresis (Bio-Rad Lab, USA) and the positive control of these enzymes (Sigma, USA) were used for identify the lysis sites which correspond the MMPs and their complexes.

The zymograms were photographed using a Sony DSC-H50 digital camera. Quantitative assessment of gelatinase activity was performed using Videodensitometer Sorbfil 2.0 software. The activities of MMP2/9 and the proMMP9/NGAL complex were calculated in arbitrary units (AU) relative to the activity of these enzymes in a standard sample, where their activity was taken as 1 AU. Pooled plasma from the control group donors was used as a standard which was obtained by mixing equal volumes of plasma samples from different donors [2]. Standard samples were frozen and stored at -80 °C.

All data are expressed as mean ± standard error of mean (SEM). Groups were compared using the one-way analysis of variance (ANOVA) followed by the Tukey test. P-values <0,05 were considered statistically significant.

Results of the study and their discussion. The 12 patients of Group 1 with moderately severe disease had a respiratory rate (RR) of 16 to 19±1 breaths per minute, oxygen saturation of 85 to 90%, no accessory muscles involved in breathing, and PEFR of 72-75% of the reference value. In Group 2 (13 patients), RR was 20 to 27±1 breaths per minute, oxygen saturation was 45-70%, accessory muscles were actively involves in breathing, and PEFR value constituted 72 to 75% of the reference value.

Recent studies suggest that IPF is associated with significant changes in gelatinase activities. On Day 1, in patients with moderately severe IPF, the activity of latent MMP9 (proMMP9) and activated MMP9 forms was similar to the Control, patients in Group 2, with a severe disease, had in general a more pronounced increase in these values (fig. 1 A, B). Such pattern remained unchanged at all stages of the study.

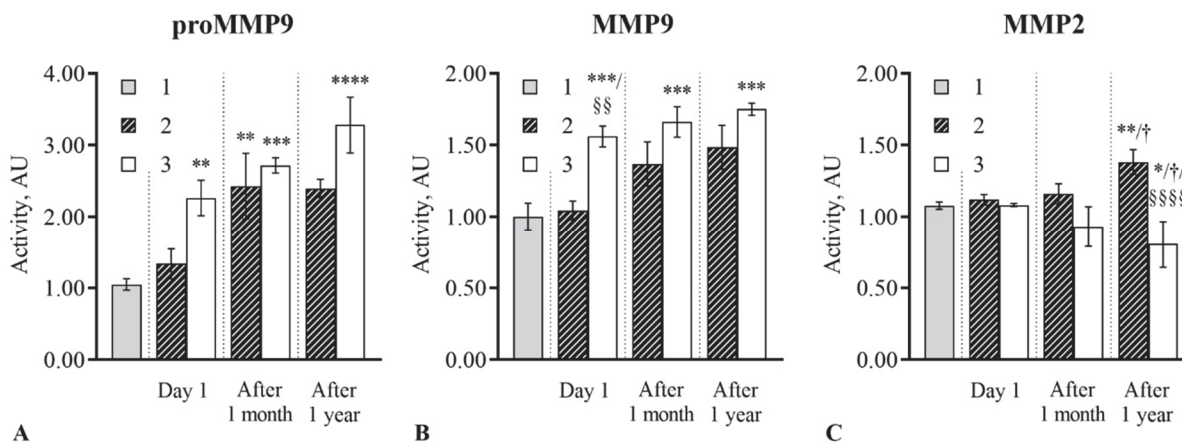


Fig. 1. Changes in proMMP9 (A), MMP9 (B) and MMP2 (C) activities in patients with idiopathic pulmonary fibrosis (IPF) depending on the disease severity. 1 – Control, 2 – Group 1 (moderately severe IPF), 3 – Group 2 (severe IPF). * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001 vs. Control, § p<0.05, §§ p<0.01, §§§§ p<0.0001 – vs. Group 1, † p<0.05, †† p<0.01 vs. values on day 1 (within the same group). AU – arbitrary unit.

In contrast, the MMP2 activity pattern appeared to be completely different. Patients in the study groups demonstrated multidirectional changes in the activity of this enzyme (Fig. 1C). An increase in MMP2 activity was observed in Group 1 during the year, while in patients of Group 2 the activity was decreasing.

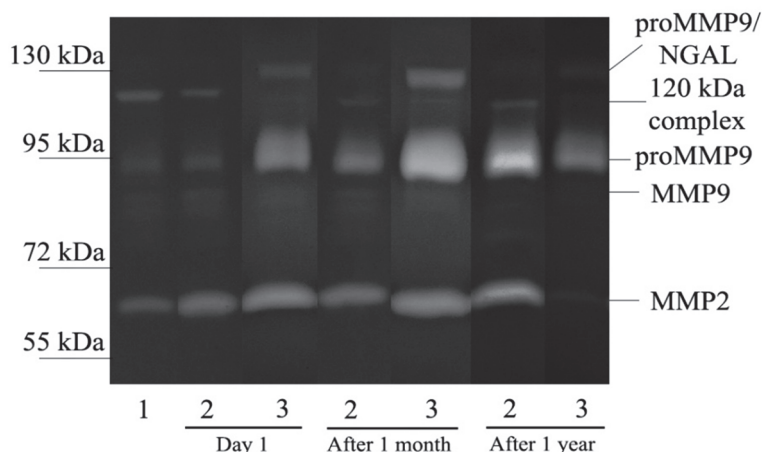


Fig. 2. Zymogram of blood plasma samples from patients with idiopathic pulmonary fibrosis (IPF) on Day 1, in one month and in one year. 1 – Control, 2 – Group 1 with moderately severe IPF, 3 – Group 2 with severe IPF.

9 out of 12 patients; while in Group 2 the proMMP9/NGAL was detected in 8 out of 13 patients, and the 120 kDa complex was found in 12 out of 13 patients.

Our results show that in the first stage, the activity of the proMMP9/NGAL complex in both clinical groups was similar to the Control (table 2).

Table 2

Activities of the proMMP9/NGAL and 120 kDa complexes in idiopathic pulmonary fibrosis of different severity (M±m)

Parameter		Control	Group 1	Group 2	
Activity, AU	proMMP9/NGAL	Day 1	0.84±0.08	1.06±0.17	
		After 1 month	–	2.50±1.50 ^{*†}	2.14±0.20 ^{**}
		After 1 year	–	2.93±0.07 ^{***††}	1.17±0.17 [§]
	120 kDa complexes	Day 1	0.91±0.10	0.62±0.12	0.47±0.10
		After 1 month	–	1.96±0.38 ^{*†††}	0.81±0.10
		After 1 year	–	2.23±0.54 ^{**†††}	1.92±1.09 ^{*†/§§§§}

Note: * p<0.05, ** p<0.01, *** p<0.001 – vs. Control, § p<0.05, §§§ p<0.001, §§§§ p<0.0001 – vs. Group 1, † p<0.05 – vs. values on Day 1 (within the same group). AU – arbitrary unit.

After one month, in Group 1 the complex activity apparently increased and then remained unchanged throughout the rest study period. Group 2 showed a different pattern: although there was a trend to an increased proMMP9/NGAL activity after one month, at one year this parameter significantly decreased with the values dropping even below the baseline level.

The baseline activity of the 120 kDa complex in patients of both clinical groups was apparently 1.5- and 2-fold below normal, respectively. However, over the course of the disease, this parameter drastically increased. After one month, in patients of Group 1 there was 3-fold increase in the activity, while in Group 2 the activity reached normal level. However, in general, after one year, patients in Group 2 apparently had a more pronounced increase in the activity (4-fold) compared with a 3.6-fold increase in Group 1.

In the present study we have demonstrated the significant changes of the gelatinases activity. Increased activity of both MMP9 forms may be explained by the fact that the main stage of the development of pulmonary fibrosis is the epithelial mesenchymal transition which includes loss of epithelial phenotype and acquisition of mesenchymal phenotype; this is also associated with an increased motility, invasiveness, acquisition of resistance to apoptosis and the ability to enhance the production of extracellular matrix components [8]. This leads to an excessive MMP9 production by transformed epithelial cells [9]. Local enhancement of the activity of both forms of gelatinase B results in excessive destruction of basement membrane proteins and increase in the total pool of type I and III collagen with a shift towards type III collagen in the lung interstitium, and a gradual increase of fibroblast clusters [14]. Therefore, a significant increase in proMMP9 and MMP9 activities at different stages of IPF can serve as an indicator of the severity of the fibrotic process, whereas multidirectional changes in the activity of MMP2 can suggest an association with the IPF stage.

Normally, NGAL is expressed in cells at very low levels. Activated neutrophils, monocytes and macrophages acquire the ability to form the proMMP9/NGAL complex (130 kDa), and the induced synthesis of NGAL is closely related to the increased expression of gelatinase B and the severity of the pathological process. Hence, significant decrease of the activity of this complex can be explained by the gradual depletion of the content of specific neutrophil granules due to phagocytic activity of neutrophils and the inability to synthesize this complex *de novo*. The role of NGAL in the proMMP9/NGAL is still under debate: the complex either enhances the stability of the proMMP9 molecule without affecting its activity, or NGAL plays the role of a nonspecific gelatinase B inhibitor and prolongs the proteolysis by preventing its autoactivation. According to the literature, the role of the proMMP9/NGAL complex in the lung pathology has only been studied in patients with chronic obstructive pulmonary disease, while its effects in IPF are unclear [5, 7].

The presence of an unusual form with a molecular weight of 120 kDa on zymogram, corresponding to another heterodimer derived from MMP9, was demonstrated by Cataldo D. et al. based on the ability of this gelatinolytic species to bind gelatin and anti-MMP9 antibody [7]. Unfortunately, lack of data on the structure and role of the 120 kDa complex, it is difficult to explain our data, so future investigations should focus on better understanding the role of this complex in the pathogenesis of IPF.

Conclusions

1. The study showed that the increase in gelatinase A and B activities in idiopathic pulmonary fibrosis was associated with the disease progression, including increased severity of the disease, worsened signs and symptoms of respiratory failure, decreased oxygen saturation, worsened spirometry parameters.

2. Increased activities of proMMP9 and MMP9 at different stages of the disease can indicate the severity of the fibrotic process, while MMP2 activity can be suggestive of its stage.

3. Changes in the activity of the proMMP9/NGAL complex reflect clinical signs and symptoms of idiopathic pulmonary fibrosis and are associated with the severity of the disease. A more favourable course of disease (Group 1) was characterized by an increased activity, which may be due to activation of the defensive mechanisms. Under conditions of the protective mechanisms depletion in Group 2, clinical signs of the disease aggravated, with the development of an expressed respiratory insufficiency associated with the subsequent poor prognosis.

4. The 120 kDa complex activity can be used as an additional criterion for evaluation of the intensity of the proteolytic process in lung tissue and the severity of IPF.

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Реферати

**НОВІ НЕІНВАЗИВНІ МАРКЕРИ ТЯЖКОСТІ
ПЕРЕБІГУ ІДІОПАТИЧНОГО ФІБРОЗУ
ЛЕГЕНЬ**

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Ідіопатичний фіброз легень є важким, неухильно прогресуючим захворюванням, з-за недостатньої специфічності ознак та мінливого характеру перебігу якого важливим є пошук додаткових неінвазивних маркерів для встановлення діагнозу, визначення ступеня тяжкості та моніторингу ефективності лікування. Метою дослідження було визначення активності желатиназ А та В, а також комплексу прожелатиназа В/ліпокалін у хворих з помірним та тяжким перебігом ідіопатичного фіброзу легень. Встановлено, що підвищення активності желатиназ пов'язано з прогресуванням захворювання. Збільшення рівня активності прожелатинази В та її активної форми на різних етапах захворювання може слугувати показником ступеня розвитку фіброзного процесу, тоді як активність желатинази А – його стадії. Зміни активності комплексу желатиназа В/ліпокалін відбивають клінічні особливості перебігу ідіопатичного фіброзу легень та пов'язані з тяжкістю захворювання.

Ключові слова: ідіопатичний фіброз легень, желатинази А та В, комплекс прожелатиназа В/ліпокалін.
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**НОВЫЕ НЕИНВАЗИВНЫЕ МАРКЕРЫ ТЯЖЕСТИ
ТЕЧЕНИЯ ИДИОПАТИЧЕСКОГО ФИБРОЗА
ЛЕГКИХ**

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Идиопатический фиброз легких является тяжелым, неуклонно прогрессирующим заболеванием, из-за недостаточной специфичности признаков и изменчивого характера течения которого важным является поиск дополнительных неинвазивных маркеров для постановки диагноза, определения степени тяжести и мониторинга эффективности лечения. Целью работы было определение активности желатиназ А и В, а также комплекса прожелатиназа В/липокалин у больных с умеренным и тяжелым течением идиопатического фиброза легких. Установлено, что повышение активности желатиназ связано с прогрессированием заболевания. Увеличение уровня активности прожелатиназы В и ее активной формы на разных этапах заболевания может служить маркером степени развития фиброзного процесса, тогда как активность желатиназы А – его стадии. Изменения активности комплекса прожелатиназа В/липокалин отображают клинические особенности течения идиопатического фиброза легких и связаны с тяжестью заболевания.

Ключевые слова: идиопатический фиброз легких, желатиназы А и В, комплекс прожелатиназа В/липокалин.
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**PECULIARITIES OF THE IMMUNE STATUS IN INDUSTRIAL WORKERS
WITH PNEUMOCONIOSIS IN COMBINATION WITH CHRONIC OBSTRUCTIVE
PULMONARY DISEASE**

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This article presents the results of the study on the status of humoral link in general immunity and functional activity of immune cells in workers of the mining and metallurgical industries with pneumoconiosis in combination with chronic obstructive pulmonary disease. It was found that in this category of patients humoral immunity indices were characterized by a significant increase in IgM (up to 4.5 g/l) and IgE (up to 465.6 IU/ml) compared with the control group, patients with pneumoconiosis and occupational chronic obstructive pulmonary disease. This indicated the formation of a pronounced "immune response" with the transformation of B-lymphocytes into plasma cells and stimulation of IgG secretion, promoting the prolongation of bronchopulmonary inflammation. Increased serum IgA (up to 2.8 g/l) is evidence of the simultaneous formation of "protective processes" in the respiratory tract. Reduction of spontaneous (up to 109.55 OU) and induced (up to 246.45 OU) activity of circulating immune complexes, as well as proliferative activity of lymphocytes (up to 1.29 OU) in the reaction of blast transformation of lymphocytes with mitogen coenzyme A increases the probability of recurrent disease. Increasing the content of complement (C3 component) to 1.24 g/l stimulates the production of histamine from mast cells and platelets that support phagocytosis, increase the permeability of vessel walls, spasm of smooth muscles, antigen-antibody reaction with the subsequent development of autoimmune processes in this category of patients.

Key words: pneumoconiosis, chronic obstructive pulmonary disease, workers, immune status.

This work is a fragment of the research project "Development of modern scientifically substantiated methods for diagnosis, treatment and prevention of pneumoconiosis in combination with chronic obstructive pulmonary disease in workers of the mining and metallurgical industry of Ukraine", state registration No. 0117U002311.

Lung diseases of occupational causation occupy a leading place in the general structure of occupational diseases and are one of the most important causes of temporary or permanent disability. There is a steady trend of increasing cases of these diseases for the first identified and the number of people who were recognized as disabled as a result [10].

In the mining and metallurgical industry, the impact on the organism of workers of industrial contaminants is primarily the cause of pneumoconiosis (PC) and chronic obstructive pulmonary disease (COPD) [7, 10]. COPD of occupational causation is a disease that occurs due to long-term exposure to

industrial contaminants and is characterized by progressive bronchial obstruction due to diffuse lesions of the bronchial mucosa, remodeling of their wall and the formation of pulmonary insufficiency [1]. Considering PC as a primary, mostly interstitial, pulmonary fibrosis, this disease is assessed as a pathological condition based on inflammation of the lung parenchyma [1, 7]. Undoubtedly, changes in general immunity determine the main pathogenetic links of PC and COPD of occupational causation, affect the features of their clinical manifestations and course [3, 4, 6].

The combination of PC and COPD in industrial workers is a problem to solve which the assessment of the state of general immunity is considered important in terms of studying the pathogenesis of these diseases [1]. Determining the state of humoral immunity and functional activity of immune cells is the basis for justification and implementation of measures for timely diagnosis, treatment and prevention of PC in combination with COPD.

The purpose of the study was to consider the indices of humoral immunity and functional activity of immune cells in mining and metallurgical workers, patients with PC in combination with COPD, as criteria for diagnosing the disease and developing measures to treat and prevent it.

Materials and methods. 118 employees of the mining and metallurgical industry with occupational lung diseases were examined. Of these, 33 patients with PC in combination with COPD, 16 patients with PC and 69 patients with COPD of occupational causation. The control group (CG) included 10 healthy workers.

The following indicators were determined by immunoturbidimetry: content of serum immunoglobulins A, M, G (IgA, IgM, IgG), in g/l, by immunochemical with electro-chemiluminescence immunoassay (ECLIA) – content of total immunoglobulin E (IgE), in IU/ml (Cobas 6000; Roche Diagnostics, Switzerland), by the method of flow cytometry using monoclonal antibodies – the number of B-lymphocytes (CD_{3}^{-} , CD_{19}^{+}), in% (flow cytometer instrument). The number and functional activity of immune cells were assessed by enzyme-linked immunosorbent assay (ELISA) (EUROIMMUM, Germany) and immunoturbidimetry (Cobas 6000, Roche Diagnostics, Switzerland). The content of circulating immune complexes (CICs) was determined: large, medium, small, in optical units, spontaneous and induced (stimulated) variant of their activity, in optical units, calculated the phagocytic index (PI) defined the C3 complement component and C4-2 complement component in g/l, the proliferative activity of lymphocytes in the leukocytes blast-transformation reaction (RBLT) with the mitogen of coenzyme A, in optical units, were also determined.

All employees provided written consent to conduct the study in accordance with the ethical principles of the Declaration of Helsinki Human Participation as an Object of Research and Awareness, with the permission of the Commission on Bioethics of the State Institution "UKRNDIPROMMED" (Protocol No. 93 of 30.04.2015).

Material processing was performed using a standard Microsoft Office Excel software package. The obtained data had a normal probability distribution and for their analysis were used mainly parametric criteria of Student and Fisher. The number of observations was sufficient to obtain unbiased estimates of the first two points: the arithmetic mean (M) and the standard deviation (m). Student's t-test was used to compare the mean values of quantitative indices under the condition of normal distribution. The significance level of $p < 0.05$ with a reliability of 95% was considered reliable.

Results of the study and their discussion. It was found that the content of IgA, compared with the control group, (table 1) was higher by 27.2% in patients with PC in combination with COPD ($p < 0.05$) and PC, in patients with COPD of occupational causation – by 13.6%. IgM content was the highest in patients with PC in combination with COPD by 4.5 times, in patients with PC and COPD, respectively – by 77.7% ($p < 0.002$) and – by 22.2%. The IgG content in CG patients was identical to that of patients with PC in combination with COPD, higher by 12.9% than in patients with PC and by 17.0% lower than in patients with COPD. The IgE content, in comparison with CG, was the highest in patients with PC in combination with COPD by 5.47 times ($p < 0.05$), in patients with PC and COPD, respectively by 34.4% and in 2.43 times ($p < 0.01$). The amount of CD_{3}^{-} , CD_{19}^{+} in CG exceeded the same indicator in patients with occupational lung pathology: in patients with PC in combination with COPD – by 28.7%, in patients with PC – by 68.8% ($p < 0.002$) and in patients with COPD – by 30.0% ($p < 0.05$).

When compared with patients with PC in combination with COPD, the IgA content was lower in patients with COPD by 12.0% and identical to patients with PC. The IgM content was lower in patients with PC – by 2.56 times, and in patients with COPD – by 3.7 times. The IgG content, on the contrary, was higher by 10.0% ($p < 0.05$) in patients with PC and lower in patients with COPD of occupational causation – by 19.8% ($p < 0.001$). The IgE content, in comparison with patients with PC in combination with COPD, was lower in patients with PC – by 4.06 times, and in patients with COPD – by 2.24 times. The relative amount of CD_{3}^{-} , CD_{19}^{+} in patients with PC in combination with COPD was the same as in patients with COPD and was by 31.1% higher than in patients with PC.

Indicators of humoral immunity in workers of the mining and metallurgical industry with occupational lung diseases (M±m)

Indices	Control group (n=10)	Patients with COPD of occupational causation		
		Pneumoconiosis in combination with COPD (n=33)	Pneumoconiosis (n=16)	COPD (n=69)
1	2	3	4	5
Immunoglobulin A (g/l)	2.2±0.2	2.8±0.2 [#]	2.8±0.4	2.5±0.1
Immunoglobulin M (g/l)	0.9±0.1	4.1±2.0	1.6±0.2 [#]	1.1±0.1
Immunoglobulin G (g/l)	12.4±0.8	12.7±0.5	14.0±0.4 [*]	10.6±0.3 ^{#/**}
Immunoglobulin E (IU/ml)	85.1±32.1	465.6±195.0 [#]	114.4±40.1	207.6±31.9 [#]
B-lymphocytes (CD3 ⁻ , CD19 ⁺) (%)	13.0±1.4	10.1±1.0	7.7±1.0 [#]	10.0±0.4 ^{#/**}

Note: # – The difference is significant compared to the control group (p<0.05); * – The difference is significant in comparison with the group of patients with pneumoconiosis in combination with COPD (p<0.05); ** – The difference is significant in comparison with the group of patients with pneumoconiosis (p<0.05).

Comparison of humoral immunity with patients with PC revealed that the IgA content in patients with COPD was lower by 12.0%, the content of IgM and IgG also, respectively – by 45.4% and – by 32.0% (p<0.001). The content of IgE, on the contrary, was lower in patients with PC than in patients with COPD – by 814%. The relative amount of CD3⁻, CD19⁺ in patients with PC was lower than in patients with COPD of occupational causation – by 29.8% (p<0.05).

Table 2.

Indices of functional activity of the cellular component of immunity in workers of the mining and metallurgical industry with occupational lung diseases (M±m)

Indices	Control group (n=10)	Patients with COPD of occupational causation		
		Pneumoconiosis in combination with COPD (n=33)	Pneumoconiosis (n=16)	COPD (n=69)
1	2	4	5	6
Functional activity of immune cells/CICs:				
– spontaneous (OU)	112.88±1.46	109.55±4.05	113.28±2.16	108.94±1.49
– induced (OU)	268.66±6.82	246.45±8.87 [#]	251.46±8.67	253.33±5.90
Phagocytic index	2.28±0.08	2.2±0.04	2.2±0.07	2.3±0.03 [*]
Proliferative activity of lymphocytes (RBLT) with the mitogen of coenzyme A (OU)	1.33±0.03	1.29±0.02	1.39±0.04	1.35±0.02 [*]
Circulating immune complexes:				
– large (OU)	10.02±2.7	9.46±1.06	8.66±1.56	7.23±0.41 [*]
– medium (OU)	73.41±8.50	89.06±1.77	89.57±2.57	82.72±1.25 ^{*/**}
– small (OU)	174.21±2.52	177.46±1.36	177.60±2.28	177.10±0.99
Complement (C3 component) (g/l)	1.16±0.07	1.24±0.04	1.15±0.04	1.21±0.02
Complement (C4-2 component) (g/l)	0.22±0.02	0.26±0.01	0.23±0.01 [*]	0.27±0.01 ^{*/**}

Note: # – The difference is significant compared to the control group (p<0.05); * – The difference is significant in comparison with the group of patients with pneumoconiosis in combination with COPD (p<0.05); ** – The difference is significant in comparison with the group of patients with pneumoconiosis (p<0.05)

The data in table 2 on the functional activity of some indicators of the cellular component of the general immunity indicate that in comparison with CG, spontaneous activity of immune cells in patients with PC in combination with COPD and COPD of occupational causation was lower, respectively – by 3.0% and by 3.5%. In patients with PC it was higher by 0.3%. The induced activity of immune cells was lower in all groups of sick workers: in patients with PC in combination with COPD – by 9.0% (p<0.05), in patients with PC – by 6.8% and in patients with COPD – by 6.0%. PI, in comparison with CG, did not differ significantly in patients with occupational lung pathology. The index of proliferative activity of lymphocytes in RBLT was lower in patients with PC in combination with COPD by 3.1%. However, in patients with PC and COPD, it exceeded the same indicator in CG by 4.5% and 1.5%, respectively. It was found that the number of CICs, compared to CG, was lower in sick workers: by 5.9% in patients with PC in combination with COPD, by 15.7% in patients with PC and by 38.6% – in patients with COPD. Another trend was found for the mean CICs, the number of which in these groups of patients was higher, respectively by 21.3%, 22.0% and 12.6%. A similar orientation was found for small CICs, the number of which was also higher than in CG, respectively by 1.8% in patients with PC in combination with COPD, by 1.9% – in patients with PC and by 1.6% – in patients with COPD. The content of complement (C3 component), in comparison with CG, in patients with

PC in combination with COPD and COPD of occupational causation was higher, by 6.9% and 4.3%, respectively. In patients with PC, this index was, on the contrary, lower by 0.9%. The complement content (C4-2 component) in sick workers was higher: in patients with PC in combination with COPD – by 18.2%, in patients with PC – by 4.5%, in patients with COPD – by 22.7% ($p < 0.05$).

When compared with patients with PC in combination with COPD, the spontaneous activity of the CICs was higher in patients with PC by 3.4% and, conversely, lowers in patients with COPD – by 0.5%. The induced CICs activity in patients of this group was lower than in patients with PC and COPD, by 2.0% and 2.8%, respectively. PI in these groups did not differ much. The index of proliferative activity of lymphocytes in RBLT in patients with PC in combination with COPD was lower than in patients with PC by 7.7% ($p < 0.05$) and by 4.5% than in patients with COPD. The content of large subpopulations of CICs in patients with PC in combination with COPD exceeded similar indices in patients with PC and COPD, by 9.2% and 30.8%, respectively ($p < 0.05$). The content of mean CICs subpopulations in these patients did not differ from patients with PC and was 7.7% ($p < 0.01$) higher than in patients with COPD. The content of small CICs subpopulations in patients with occupational lung diseases did not differ significantly. Compared with patients with PC in combination with COPD, the complement content (C3 component) in patients with PC was lower by 7.8%, and in patients with COPD of occupational etiology – by 2.5%. The content of the C4-2 complement component in this category of patients exceeded the same indice in patients with PC by 13.9% ($p < 0.05$) and, conversely, was lower than in patients with COPD by 3.8%.

In PC patients, spontaneous CICs activity was by 4.0% higher than in COPD patients, however, induced CICs activity was lower by 0.7%. PI did not differ significantly in patients with PC and COPD, the proliferative activity of lymphocytes in RBLT in patients with PC was higher than in patients with COPD of occupational causation by 2.9%. The content of different CICs subpopulations in patients with PC was higher than in patients with COPD: large – by 19.7%, medium – by 8.3% ($p < 0.02$), small – by 0.3%. The complement content in patients with PC was lower than in patients with COPD, respectively by 5.1 % – C3 component and 17.4 % ($p < 0.002$) – C4-2 component.

Studies have shown that humoral immunity indices of workers in the mining and metallurgical industries with PC in combination with COPD are characterized by a significant increase in IgM: by 4.5 times compared to CG, from 2.6 to 3.7 times in comparison with patients with PC and COPD of occupational causation with a simultaneous increase in IgE – by 5.4 times compared to CG, from 2.2 to 4.1 times in comparison with patients with PC and COPD of occupational causation. These changes indicate the presence of processes for mechanisms formation on the expressed "immune response" with simultaneous transformation of B-lymphocytes into plasma cells and stimulation of IgG secretion [5]. That is, the invented shifts of the humoral link of the general immunity stimulate the prolongation of chronic, mainly infectious, bronchopulmonary inflammation. An increase in IgE in the blood indicates the "trigger" of allergic reactions, which are realized by the tendency to develop bronchial hyperreactivity and subsequent atopy. Increased serum IgA forms "protective processes" in the respiratory tract. There is a tendency to decrease the spontaneous and induced activity of the CICs and the proliferative activity of lymphocytes in RBLT, which is evidence of chronic systemic inflammation and the formation of a tendency to its recurrent course. Increased complement (C3 component) is a predictor that stimulates the production of histamine from mast cells and platelets, supporting phagocytosis, increasing vascular permeability, increasing smooth muscle spasm, leukocyte chemotaxis and antigen-antibody reaction [2]. An increase in the complement content (C4-2 component) is a sign of a significant risk of occurrence and progression of infectious inflammation in sick workers. The obtained data expand the idea of the pathogenetic mechanisms of PC formation in combination with COPD and supplement the results of previous studies on the pathogenesis of dust pathology of the lungs in industrial workers [2, 5, 9].

Conclusions

1. According to the results of the study it was established that at the present stage an increase in IgM and IgE, which is a sign of high activity of chronic inflammation in the bronchopulmonary system with the presence of an allergic component, is a specific feature of the state of the humoral part of the immune system in workers of the mining and metallurgical industry with PC in combination with COPD.

2. In terms of functional activity of immune cells in patients with PC in combination with COPD, the tendency to decrease the spontaneous and induced activity of CICs and proliferative activity of lymphocytes in RBLT is a predictor of chronic systemic inflammation and the formation of predisposition to its recurrent course.

3. Increased complement content (C3 component) stimulates the activation and subsequent hyperproduction of inflammatory mediators, initiation of autoimmune mechanisms that cause prolongation of systemic inflammation with a predisposition to its infectious complications.

Prospects for further research lie in the fact that the obtained data are an important component in further research aimed at developing a scientifically sound system of measures for timely diagnosis, treatment and prevention of PC in combination with COPD in workers of the mining and metallurgical industries.

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Реферати

ОСОБЛИВОСТІ ІМУННОГО СТАТУСУ У ПРОМИСЛОВИХ ПРАЦІВНИКІВ, ХВОРИХ НА ПНЕВМОКОНІОЗ У ПОЄДНАННІ З ХРОНІЧНИМ ОБСТРУКТИВНИМ ЗАХВОРЮВАННЯМ ЛЕГЕНЬ

Рубцов Р.В.

В статті викладено результати досліджень щодо вивчення стану гуморальної ланки загального імунітету та функціональної активності імунних клітин у працівників гірничорудної та металургійної промисловості, хворих на пневмокониоз у поєднанні з хронічним обструктивним захворюванням легень. Встановлено, що у цієї категорії хворих показники гуморального імунітету характеризуються суттєвим збільшенням IgM (до 4,5 г/л) та IgE (до 465,6 МО/мл), у порівнянні з контрольною групою, хворими на пневмокониоз та хронічне обструктивне захворювання легень професійної етіології, вказуючи на формування вираженої «імунної відповіді» з трансформацією В-лімфоцитів у плазматичні клітини та стимуляцією секреції IgG, сприяючи пролонгації бронхолегеневого запалення. Збільшений вміст у сироватці крові IgA (до 2,8 г/л) є свідченням одночасного формування «захисних процесів» у дихальних шляхах. Зменшення спонтанної (до 109,55 опт.од.) та індукованої (до 246,45 опт.од.) активності циркулюючих імунних комплексів, а також проліферативної активності лімфоцитів (до 1,29 опт. од.) у реакції бластної трансформації лімфоцитів з мітогеном коензиму А посилює вірогідність рецидивуючого перебігу хвороби. Збільшення вмісту комплекменту (С3 компоненту) до 1,24 г/л стимулює продукцію гістаміну з опасистих клітин та тромбоцитів, які підтримують фагоцитоз, посилюють проникність стінок судин, спазм гладкої мускулатури, реакцію антиген-антитіло з подальшим розвитком аутоімунних процесів у цієї категорії хворих.

Ключові слова: пневмокониоз, хронічне обструктивне захворювання легень, працівники, імунний статус.

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ОСОБЕННОСТИ ИМУННОГО СТАТУСА У ПРОМЫШЛЕННЫХ РАБОЧИХ С ПНЕВМОКОНИОЗОМ В СОЧЕТАНИИ С ХРОНИЧЕСКИМ ОБСТРУКТИВНЫМ ЗАБОЛЕВАНИЕМ ЛЕГКИХ

Рубцов Р.В.

В статье изложены результаты изучения гуморального звена общего иммунитета и функциональной активности иммунных клеток у рабочих горнорудной и металлургической промышленности с пневмокониозом в сочетании с хроническим обструктивным заболеванием легких. Установлено, что у этой категории больных увеличено содержание IgM (до 4,5 г/л), IgE (до 465,6 МО/мл), в сравнении с контрольной группой, больными пневмокониозом и хроническим обструктивным заболеванием легких профессиональной этиологии, указывая на формирование выраженного «иммунного ответа» с трансформацией В-лимфоцитов в плазматические клетки, стимуляцией секреции IgG, способствуя пролонгации бронхолегочного воспаления. Повышенное содержание в сыворотке крови IgA (до 2,8 г/л) отражает формирование «защитных процессов» в дыхательных путях. Уменьшение спонтанной (до 109,55 опт.ед.) и индуцированной (до 246,45 опт.ед.) активности циркулирующих иммунных комплексов, а также пролиферативной активности лимфоцитов (до 1,29 опт. ед.) в реакции бластной трансформации лимфоцитов с митогеном коэнзима А, усиливает вероятность рецидивирующего течения болезни. Увеличение содержания комплекмента (С3 компонента) до 1,24 г/л стимулирует продукцию гистамина тучными клетками и тромбоцитами, вызывая фагоцитоз, усиливая проницаемость стенок сосудов, спазм гладкой мускулатуры, реакцию антиген-антитело с развитием аутоиммунных процессов у этой категории больных.

Ключевые слова: пневмокониоз, хроническое обструктивное заболевание легких, рабочие, иммунный статус.

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PECULIARITIES OF HEART RATE VARIABILITY INDICATORS IN TRACK-AND-FIELD ATHLETICS WITH MESOMORPHIC SOMATOTYPE

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Disruption of autonomic-vegetative homeostasis is a direct path to the sports, especially cardiac, pathology appearance. Membership of the mesomorphic somatotype does not reduce the differences in the magnitude of cardiointervallographic indices between groups of athletes and non-athletes. We established a certain imbalance in the regulation of heart rhythm of athletes. According to the results of spectral indices, parameters of variational pulsometry and vegetative homeostasis ambiguous data were obtained, characterizing the influence on the athletes of the parasympathetic and sympathetic borders, which indicates the adaptation-regulatory mechanisms tension on them.

Keywords: cardiointervallography, mesomorphic somatotype, field-and-run athletes, young men.

The study is a fragment of the research project "Indicators of heart rate variability in virtually healthy boys and girls with different types of hemodynamics", state registration No. 0118U003452.

Nowadays, athletes face with high professional requirements in the process of achievement their goals and desires. The development of physical fitness resistant starts to form in athletes that practice sports from their childhood, which, from the point of view of some authors, is marked by constitutional features [5]. Pphysical activity should be gradual and meet age-appropriate physiological standards. Appropriate balance between stress and recovery is significantly important for athletes to achieve continuous high-level results [12]. That regulations related to the deterioration of health indicators of people involved in sports, which were not familiarized in time to the plan of the training process, can lead to the development of maladaptation in their bodies [2]. The main problems of the development of modern sport prevention medicine are in the lack of data obtained on the regime of the training process of athletes with different anthropometric indicators and constitutional types. This question is extremely urgent, due to the fact that studying these indicators will give the opportunity to introduce individual athlete's training process evaluation maps. Such kinds of maps will to predict the level of willingness to perform the load without the health disorders occurrence [6].

Pronounced impacts on athletes, disorders of the autonomic nervous system have. Moreover, they are believed to be one of the common signs of health disorder and manifestation of formation to nosological and pathological changes of the body [8]. Detection of abnormalities in the regulation of the cardiovascular system that was taken in time, may not only prevent pre-pathological conditions, but also avoid them.

Currently, the subject of sports, physical fitness and adaptive abilities of the person to trainings has become central. Elite athletes use all their energy to achieve results and become extremely exhausted. Thereby, the question of their life expectancy arises, moreover, determination of the relationship between long-term energy training and survival appear. Such kinds of changes may be adaptive, or they can lead to heart complications. Currently, regular medical examinations of athletes are being conducted with registration of electrocardiographic, echocardiographic and other indicators, which generally reflects the state of health and morbidity of young people. Specific attention should be paid to both aspiring athletes and athletes with a high level of athletic skill. The most simple and non-invasive method in the study of cardiac activity is the study of heart rate variability [13].

The study on the adaptation of athletes has not been sufficiently studied [4], that does not allow to carry out the analysis of the variability of cardiac rhyme for the detection of maladaptation and to nosological disorders of cardiac activity and determines the relevance of this study deeply.

The purpose of the study was to establish changes in statistical and spectral indices of heart rate variability and indices of variational heart rate and vegetative homeostasis in athletes with the mesomorphic type of constitution.

Materials and methods. The study involved young men (from 17 to 21 years old). The control group consisted of 80 young men who did not play sports and were almost healthy at the time of the examination. The conclusion was made after detailed clinical and laboratory study that were performed at the research laboratory of functional morphology and genetics of the research center in National Pirogov Memorial Medical University, Vinnytsya. All examined were under: chest radiography, spirometry, echocardiography, electrocardiography, tetrapolar rheocardiography, sonography of the thyroid gland and parenchymatous organs of the abdominal cavity, kidney, bladder, determination of major biochemical levels and thyroid hormone levels.

Furthermore, athletes with high levels of sportsmanship (from the first adult category to masters of sports) who had sports loads of cross-country running with maximum (jogging at 100, 200, 110 m hurdles) and submaximal (jogging at 400 and 800 m) intensity of work, were surveyed.

All athletes have been engaged in sports for at least 3 years, the average sports experience in this group was 6.78 ± 2.24 years. At the time of the examination, all athletes were in the preparatory period of the training cycle. Diagnosis was performed in 12 hours after exercising. All athletes went through chest radiography, spirometry, echocardiography, electrocardiography, tetrapolar reocardiography. In cases where unconventionalities from the norm were found, in particular high blood pressure, arrhythmias, insufficiency of two-leaf and three-leaf valves and severe myocardial hypertrophy, athletes did not participate in the further study. Thus, the study group was formed out of 50 athletes.

We studied heart rate variability on the cardiac computer diagnostic complex. The rhythmogram was fixed while recording the electrocardiogram in the second standard lead for 5 minutes. A pneumogram was recorded at the same time, using a nasal thermistor. Cardiac rhythm data were analyzed using a computer program of a certified cardiac diagnostic complex. The results were evaluated according to the recommendations of the European and North American Cardiac Association [14]. The definition of indicators of variational heart rate included: moda - the average value of R-R interval (MO), the moda amplitude (AMO), the average (NNM), the minimum (MIN) and the maximum (MAX) meaning of R-R intervals (including abnormal R-R intervals); variation range (VR). Among the statistical indicators of heart rate variability was determined: Standard deviation of NN intervals (SDNN), root mean square of successive R-R interval differences (RMSSD); percentage of successive RR intervals that differ by more than 50 ms (pNN50). According to the Baevsky method, the indices of vegetative homeostasis were determined: the voltage index of regulatory systems (IN); index of vegetative balance (IVR); vegetative rhythm rate (VPR). During the HRV spectral analysis, the following frequency ranges were determined: low frequency (VLF), Low Frequency (LF), High Frequency (HF). Power ratios in the low and high frequency ranges were calculated (LF/HF).

Somatotypes were determined using a calculated modification of the Heath-Carter method [10]. After somatotypological analysis, it was found that the largest number of athletes and control group members belonged to the mesomorphic constitution type: 29 athletes and 23 non-athletes. Statistical processing of the results was performed using the "STATISTICA 5.5" package (license number AXXR910A374605FA). The distributions for each variation were evaluated by the Shapiro-Wilk methodic, the mean of average and standard deviation for each trait and the significance of the difference of values were determined according to the Mana-Whitney U test.

Results of the study and their discussion. Analyzing the peculiarities of cardiac rhythm indices in highly qualified athletes and young people from the control group, who were not engaged in sports and were practically healthy by the results of the complex medical examination, it is necessary to note the significant heterogeneity of cardiointervalographic indicators in both observation groups, which is indicated by the quadruple standard values one third of the average in the sample.

It is necessary to dwell on the statistical indicators of heart rate variability. We determined that athletes had significantly less standard deviation of normal R-R intervals ($p < 0.05$) than participants of control group. RMSSD did not significantly differ ($p > 0.05$) between the comparison groups. The pNN50 value (%) of track-and-field athletes was significantly higher ($p < 0.01$) comparing to somatotype of non-sport youngsters. We have found out that individual variations in heart rate variability had significant differences between adolescents belonging to the mesomorphic somatotype, but differed in the level and specificity of motor activity. It was establish that the mean and maximum R-R interval in athletes were drastically greater than in control group (in both cases, $p < 0.05$). And the moda did not have considerable differences between comparison groups, but it should be noted that the arithmetic mean of this indicator of variational heart rate in the group of athletes-mesomorphs is greater than that of non-athletes of the same constitutional type. The moda amplitude and the minimum R-R interval were not significantly different between athletes and control group. The variational span of the variations of the pulsometry in the group of athletes was significantly smaller than that of the non-athletes (table 1).

Most spectral indicators of heart rate variability in athletes with high levels of sportsmanship were smaller compared to non-athletes. Thus, the total recording power in all ranges in athletes was 31.55% fewer ($p < 0.05$) than in the control group. Despite the lower average statistic sings of VLF in the track-and-field athletes group, the difference in the magnitude of this spectral indicator between the comparison groups was not considerable. We noted that the power in the low-frequency range of athletes was for 37.73% lower ($p < 0.05$) compared to non-athletes. Power in the range of high frequencies in the group of

athletes had lower values by 45.96% ($p < 0.05$), than in the control group. The power ratios in the low and high frequency ranges did not differ drastically between the groups of athletes and non-athletes (table 1).

Table 1.

Features of heart rate variability indicators in athletes with mesomorphic somatotype

Indices	The studied groups	$M \pm \sigma$	p
Statistical indices of heart rate variability			
SDNN (ms)	Non-athletes	92.50±24.21	<0.05
	Athletes	70.56±22.72	
RMSSD (ms)	Non-athletes	90.91±44.00	>0.05
	Athletes	85.60±38.21	
PNN50 (%)	Non-athletes	37.55±14.53	<0.01
	Athletes	51.95±15.71	
Indices of variational heart rate			
MO (ms)	Non-athletes	1.030±0.172	>0.05
	Athletes	1.086±0.143	
AMO (%)	Non-athletes	34.70±13.20	>0.05
	Athletes	34.21±11.58	
NNM (ms)	Non-athletes	1.013±0.141	<0.05
	Athletes	1.088±0.121	
MAX (ms)	Non-athletes	1.223±0.180	<0.05
	Athletes	1.342±0.154	
MIN (ms)	Non-athletes	0.794±0.126	>0.05
	Athletes	0.828±0.140	
VR (ms)	Non-athletes	0.351±0.069	<0.05
	Athletes	0.338±0.067	
Spectral indices of heart rate variability			
FD (ms ²)	Non-athletes	13737.9±5070.1	<0.05
	Athletes	9403.3±4760.3	
VLF (ms ²)	Non-athletes	3938.2±1965.2	>0.05
	Athletes	3679.9±1799.3	
LF (ms ²)	Non-athletes	3949.9±1988.1	<0.05
	Athletes	2459.8±1703.1	
HF (ms)	Non-athletes	5644.9±2718.6	<0.05
	Athletes	3050.4±1885.8	
LF/HF	Non-athletes	0.777±0.341	>0.05
	Athletes	0.945±0.545	
Indices of vegetative homeostasis estimation by Baevsky's method			
IN	Non-athletes	50.45±22.89	>0.05
	Athletes	50.58±24.49	
IVR	Non-athletes	90.67±30.48	<0.05
	Athletes	131.9±61.33	
VPR	Non-athletes	3.678±1.388	<0.05
	Athletes	2.929±0.861	

After collation of vegetative homeostasis indices, we establish that the voltage index of regulatory systems was practically indistinguishable between group of athletes and control one ($p > 0.05$). Having estimated the indices of vegetative homeostasis, we found that the voltage index of regulatory systems is practically indistinguishable between groups of athletes and control ($p > 0.05$). The index of vegetative balance of athletes was 31.26% higher ($p < 0.05$) compared to non-athletes. And the vegetative rhythm rate indicator of athletes with mesomorphic somatotype, on the contrary, was significantly lower ($p < 0.05$) than in their peers of the control group (Table 1).

The constitutional features of the morpho-functional parameters of the human body have been proven by many studies [11]. Constitutional conditionality of cardiovascular parameters [7, 3, 6, 9] and regulation of the heart are not an exception to this list [2, 8]. It is established that the activity of the mechanisms of self-regulation of the parasympathetic autonomic nervous system is higher in the men of mature age of the mesomorphic somatotype than in the representatives of the ecto-mesomorphic type [1]. But the question remains, whether the differences in cardiovascular indices persist in representatives of one somatotype with different levels of locomotor activity. The answer to this question can be obtained by comparing the magnitude of individual indicators between groups of non-sports and highly professional athletes, whose bodies were, for a long time, affected by intense workloads that were caused by a specific

sport requests. That is, the question arises: what is the primary and the most important course - belonging to a particular constitutional type or specificity of a particular sport activity? Analyzing indicators of central hemodynamics in volleyball players of the Super League of Ukraine, it was found that volleyball players with mesomorphic and intermediate somatotypes had significantly greater stroke and minute volumes, left ventricular capacity and volumetric blood flow speed, than non-sports girls with the same kind of somatotype; in persons with ectomorphic component somatotype prevalence, there are no differences in the amount of hemodynamic parameters depending on sports activity [9]. Studying the indicators of peripheral hemodynamics in the representatives of male adolescents, who professionally engaged in volleyball, athletics and wrestling, it was found that only athletes of mesomorphic somatotype had the most significant differences in the magnitude of rheovasographic parameters of the thigh, which are concomitant engaged in sports [7].

Significant differences in cardiac rhythm variability were also found between groups of mesomorph athletes and adolescents not involved in the sports of the mesomorphic somatotype, in our research. In particular, the SDNN, which characterizes changes in intervals between heartbeats of normal sinus rhythm and indirectly indicates heart rate, is considerably lower in the group of athletes. The value of this indicator, according to most authors [4, 9-14], reflects the cumulative effect of the influence of the sympathetic and parasympathetic parts of the autonomic nervous system, so smaller SDNN values may be indicative of a more regulated heart rhythm and a more pronounced effect of the parasympathetic nervous system. Higher values of SDNN in non-athletes may point out the stress of humoral regulation and central oscillator activity. The value of RMSSD had no significant differences between the comparison groups, a slight increase in its average values in the control group can be explained by a more pronounced parasympathetic influence on the heart. This is most likely a reflection of sinus arrhythmia associated with breathing, which is more pronounced in untrained individuals. The significantly higher PNN50 value in the group of athletes confirms their overwhelming influence on the variability of the heart rate of the parasympathetic nervous system [4, 8].

According to the results of the variations of the pulsometry an ambiguous data were gotten. Those outcomes characterized the influence on the athletes of the parasympathetic and sympathetic contours. Thus, the magnitude of moda, the amplitudes of the moda and the minimum R-R interval did not differ drastically between the comparison groups, but it should be noted that the average values of MO in the group of athletes were greater than in the control. We consider this feature as a manifestation of sympathicotonia, which may be a sign of first-degree overtraining in athletes. In addition, we found that the average value of the R-R interval maximum value of the R-R intervals in mesomorphic athletes was statistically noteworthy, and therefore the influence of the parasympathetic department of the autonomic nervous system in them was more considerable [8], than in the group of control. The variational range in both groups showed a moderate preference for the parasympathetic link.

The same ambiguous data were obtained in the analysis of spectral indices. Yet, the total recording power across all ranges in athletes was significantly less than in the control, indicating less variability in heart rate athletes. Some authors have suggested that a decrease in heart rate variability was evidence of increased sympathetic activity [4, 13], in addition, it has been suggested that with a prominent increase of heart rate and a decrease of heart rate variability, sudden deaths were more commonly reported in professional sports, that is why we considered lower values of total recording power in all ranges in athletes as a sign of functional adaptation. to overtraining and to acute or chronic overstrain of the cardiovascular system. In the VLF, LF, HF control group, all three units of cardiac regulation (humoral, sympathetic, and parasympathetic) were dominated. Thus, significantly greater importance in adolescents who did not engage in dispute, the mid-frequency spectral range indicated a more pronounced sympathetic-parasympathetic regulation of vascular tone. In previous studies [7, 9] central and peripheral hemodynamics showed that athletes had vascular tone greater than non-athletes, and depending on the development of skeletal muscle, we can assume that they have a more stable figure. The HF spectral index, which reflects the power at high frequencies (0.15-0.4 Hz), was higher in the control group boys and was a confirmation of greater vagal control in their heart rhythm and more pronounced influence on the heart rhythm of respiratory movements. The LF/HF ratio was more important in athletes in favor of the predominance of the parasympathetic nervous system.

In both groups, the comparison of the values of the Bayevsky index, which reflects the level of tension (stress) of the regulatory mechanisms, the activity of the mechanisms of sympathetic regulation, the state of the central circuit, corresponded to the values of rest and characterized the equilibrium of the sympathetic nervous system. Indicators of IVR, which were significantly higher in athletes, indicated a shift in the autonomic equilibrium index towards the activity of the sympathetic autonomic nervous system, which could also indicate signs of central nervous system overstrain occurring under the influence of

systematic high physical activity. The value of VPR indicated that the athlete's autonomic rhythm was lower, and therefore the vegetative balance was more shifted to the parasympathetic side.

Thus, we found a certain imbalance in the regulation of heart rhythm in athletes. Disruption of vegetative homeostasis leads to the development of pre-pathological and pathological conditions, especially in the body of athletes. Most scientists [2, 8, 13] associate cardioprotective effect of physical activity influence with the tendency of parasympathetic activity increase, which reflects in the vagus-associated cardiointervalographic indexes raise. The autonomic regulation of the heart rhythm has the character of complex reciprocal interaction of the sympathetic and parasympathetic departments of the autonomic nervous system and has a direct dependence on the type of sports activity of an individual.

Conclusions

1. Despite belonging to one constitutional type - mesomorphic, we have found significant differences in cardiointervalographic parameters between groups of athletes and non-athletes.
2. Significant changes in statistical indicators of heart rate variability were revealed: in athletes of mesomorphic somatotype smaller values of SDNN and greater values of PNN50, which confirms their predominant influence on the heart rate variability of the parasympathetic nervous system, compared with non-athletes of the same somatotype.
3. According to the results of variational pulsometry and spectral indices, ambiguous data were obtained, characterizing the influence on the athletes of the parasympathetic and sympathetic lineaments.
4. Estimation of the vegetative homeostasis sings indicates an imbalance in the regulation of heart rhythm of athletes, which is the evidence the tension in them of the adaptive-regulatory mechanisms.
5. The use of the cardiointervalographic method provides an opportunity for early recognition of signs of regulatory systems overstretching and inadequacy of the athlete's reaction to training and competitive loads, which will allow for the introduction of correctness in sports activities.

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Реферати

ОСОБЛИВОСТІ ПОКАЗНИКІВ ВАРІАБЕЛЬНОСТІ СЕРЦЕВОГО РИТМУ У ЛЕГКОАТЛЕТІВ МЕЗОМОРФНОГО СОМАТОТИПУ

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Порушення вегетативного гомеостазу – це прямий шлях до виникнення спортивної патології, особливо до кардіальної. Належність до мезоморфного соматотипу не зменшує відмінностей у величині кардіоінтервалографічних показників між групами

ОСОБЕННОСТИ ПОКАЗАТЕЛЕЙ ВАРІАБЕЛЬНОСТИ СЕРДЕЧНОГО РИТМА У ЛЕГКОАТЛЕТОВ МЕЗОМОРФНОГО СОМАТОТИПА

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Нарушение вегетативного гомеостазу - это прямой путь к возникновению спортивной патологии, особенно кардиальной. Принадлежность к мезоморфному соматотипу не уменьшает различий в величине кардиоинтервалографических показателей между группами легкоатлетов и

легкоатлетів і неспортсменів. Нами виявлений певний дисбаланс в регуляції ритму серця у легкоатлетів. За результатами спектральних показників, параметрів варіаційної пульсометрії та вегетативного гомеостазу отримано неоднозначні дані, що характеризують вплив на легкоатлетів парасимпатичного і симпатичного контурів, що свідчить про напруження у них адаптаційно-регуляторних механізмів.

Ключові слова: кардіоінтервалографія, мезоморфний соматотип, легкоатлети, юнаки.

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неспортсменов. Нами обнаружен определенный дисбаланс в регуляции ритма сердца у легкоатлетов. По результатам спектральных показателей, параметров вариационной пульсометрии и вегетативного гомеостаза получены неоднозначные данные, характеризующие влияние на легкоатлетов парасимпатического и симпатического контуров, что свидетельствует о напряжении у них адаптационно-регуляторных механизмов.

Ключевые слова: кардиоинтервалография, мезоморфный соматотип, легкоатлеты, юноши.

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OPTIMIZED MEASURES FOR CORRECTION OF DENTAL STATUS IN PROFESSIONAL ATHLETES

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The clinical state of the oral cavity, the functional state of the salivary glands, and the effectiveness of therapeutic and preventive measures using propolis-based medicine (Balsam Pomegranate) were studied in 122 professional wrestlers aged 18 to 34 years. In the course of our observations it has been ascertained fact in digital values and a significant reduction of unfavorable background unstimulated salivary secretion in subjects athletes at the studied length of stay in prolonged intense psycho-emotional and physical condition. Status of oral hygiene and periodontal tissues in the study group of athletes who have practically healthy periodontium, deteriorated after an intensive training process at almost 1.5 times. Preparation "Balsam Pomegranate" has a very important, especially with the preventive point of view, antiinflammatory, organoleptic properties, as based on the natural stimulation help to improve the hygienic condition of the oral cavity, the state of periodontal tissues, as well as a pronounced stimulation of salivation and increasing salivary flow rate.

Keywords: periodontitis, saliva, hygiene, intense exercise, prevention.

The work is a fragment of the doctoral dissertation "Development of differentiated approaches to the prevention of inflammatory periodontal diseases in professional athletes".

Based on the results of clinical and epidemiological studies, the importance of close cooperation of specialists of various fields, including a dentist, a therapist, a sports doctor, a cardiologist, as well as a personal trainer, was determined for the successful development and increase of the effectiveness of medical and preventive measures in the field of sports medicine, which ultimately creates the conditions for choosing the optimal therapeutic tactics and further conducting a professional athlete with pathologies of various organs and systems of the body [1, 3, 7]. At the same time, according to scientists, the development and implementation of practical recommendations to improve the general condition, quality of life, health, increase efficiency with the subsequent increase in sports performance of the highest achievements should be carried out taking into account the period and correction of the training process, as well as the degree of development and pathogenicity of chronic infection [2, 6].

The most frequent periods of the training cycle, favorable for the occurrence and development of pathological processes in the periodontal soft and hard tissues among professional athletes, as indicated in some literary sources, are the pre-competitive and competitive periods, because during these periods the number of cases of occurrence and exacerbations of inflammatory diseases in periodontal tissues sharply increases, they against the background of deterioration of vital physical and emotional indicators, can cause certain disorders in the organs of the gastrointestinal tract, in the state of the local immune system, and thus increase the severity of somatic pathology [4, 5, 8].

The purpose of the work was increasing the level of dental care for professional athletes on the background of intense physical and psycho-emotional stress with the use of biologically neutral medicine.

Materials and methods. The prevalence of periodontal disease among 200 athletes aged 18–25, 26–30 and 31–38 years was determined using the CPITN (Community Index of Periodontal Treatment Need, WHO, 1980). Also, 122 professional athletes-wrestlers aged from 18 to 34 years old took part in these scientific studies to study the functional state of the salivary glands and the effectiveness of therapeutic and preventive measures. Salivation indicators were expressed in ml / min. Then a comparative statistical analysis was obtained by comparing the amount of saliva excreted in all experimental groups, excreted at rest and after completion of intense physical exertion and complex therapy.

Clinical studies included: an assessment of the hygienic state of the oral cavity (according to Quigley and Hein (1962): Silness-Loe plaque index (IPI Silness-Loe, 1964) and periodontal tissue condition (iodine number of Svrakova, 1962), Bleeding index (Muhlemaim, 1971, Cowell I., 1975). In order to stimulate salivation and improve the oral hygiene in the examined athletes, the efficacy of using a propolis-based medicine (Pomegranate balm) was evaluated (composition: propolis, extracts of *Leuzea carthamoides*, *Rhodiola rosea*, Manchurian aralia, *Abies*) in the main Group I (n=16). In the control Group II (n=15) in the complex treatment the traditional antiseptic 0.05% chlorhexidine bigluclate was used.

To study the qualitative and quantitative composition of the microflora of the oral cavity were used: 5% Blood Agar for determining the total level of microbial contamination of the oral cavity, vitelline - salt agar for staphylococci, Glucose Broth and Mitis Salivarius Agar for streptococci, for *Candida* fungi - CandiSelect agar (Boi-Rad, France). The samples were immediately placed into the Stuart's transport environment and sent to a laboratory for further research.

The results of the study were processed by the method of variation statistics. To characterize the group of homogeneous units, their arithmetic mean values (M), its standard error (SE) - (m) and the range of changes (min-max) were determined. For statistical data processing, the nonparametric criterion U (Wilcoxon-Mann-Whitney) and the parametric data (Student T-test) were used as a method for assessing differences in indicators. We used signed-rank test to compare paired data, statistically significant *p*-values were considered *p*<0.05. Statistical processing of the obtained data was carried out on a personal computer using modern software - Microsoft Excel 2007 spreadsheet editor and application program Statistica 7.0.

Results of the study and their discussion. In the process of clinical studies of the oral cavity in professional athletes, a high percentage of the prevalence and intensity of inflammatory and destructive diseases of periodontal tissues was revealed, the main cause of the development and chronicity of these was the intense and prolonged physical activity (Table 1).

Moreover, a decrease in the number of persons with a healthy periodontal tissue was observed even in younger age groups. Thus, the average value of the CPITN index for the frequency of occurrence of intact periodontium in examined professional athletes aged 18–25 years was 15.38±4.48%, and for athletes belonging to older age groups, the number of intact periodontal soft tissues amounted to even smaller values - 9.3±3.36% and 5.00±2.81%, among athletes aged 26–30 and 31–38, respectively, that is, with an increase in age indicators, a pronounced dynamics was recorded in decline of indicators.

Table 1

CPITN index among professional athletes

Age groups	Number of examined	The number of examined, %				
		Healthy periodontium	Bleeding	Tartar	Periodontal pockets	
					4–5 mm	More than 6 mm
18–25	65	15.38±4.48	21.54±5.10	49.23±6.20	13.85±4.28	-
26–30	75	9.33±3.36	12.00±3.75	37.33±5.59	32.00±5.39	9.33±3.36
31–38	60	5.00±2.81	6.67±3.22	41.67±6.36	35.00±6.16	11.67±4.14
Total	200	10.00±2.12	13.50±2.42	42.50±3.50	27.00±3.14	7.00±1.80

Note: Data are presented as n (%) of CPITN: Community Periodontal Index for Treatment Needs

When studying personal data and comparing with the results of the studies, it was revealed that the frequency of inflammatory periodontal diseases is directly correlated with sports experience, as well as with the qualifications of athletes. The salivation rate in almost all the examined professional athletes against the background of maximum physical exertion and stress disorders which are characteristic of the pre-competitive and competitive periods was low and indicated severe form of hyposalivation. In the presence of certain diseases of the gastrointestinal tract in individual cases, athletes with a pronounced and extremely low degree of secretion of salivary glands and salivation - xerostomia were detected.

The average salivary flow rate revealed almost in all groups of examined athletes testified to hyposalivation in the oral cavity. The main difference among professional athletes who are already in a psycho-emotional impaired state during intense training on the background of chronic stress and sometimes depression was that, unlike people who do not play sports professionally, there the amount of excreted saliva slightly increased in stimulating salivation. In the course of our observations, was found the fact of the digital values of a significant and unfavorable decrease in the unstimulated saliva secretion in the examined athletes during a prolonged intense psycho-emotional and physical state (2.14±0.020 ml / min in athletes with a healthy periodontal tissue without stimulation and before the start of training and 1.74±0.016 ml / min respectively after their completion, *p*<0.001).

To study the state of salivary glands, this stage of the research was carried out in several stages: at the initial stage of clinical observations, the dynamics of indicators changes was studied depending on the

nature of the therapeutic measures used during between the main stages of the training cycle (using data from a previous survey), at a later second stage, a comparative evaluation of the results obtained before training, as well as treatment and immediately after and after their finishing. The results are presented in the table below.

As can be seen from the obtained tabular data, in the examined athletes with an intact periodontium after stimulation, the salivation rate decreased a few hours after the training completion from 3.2 ± 0.040 ml / min to 2.56 ± 0.028 ml / min; in professional athletes with pathological changes in soft periodontal tissue, the salivation rate compared to the data before intense physical exertion also decreased and by the end of the study approximately was 1.34 ± 0.019 ml / min versus 1.68 ± 0.025 ml / min before training ($p < 0.001$). It is important to note that at all stages of the observations at the intergroup comparative assessment, the differences turned out to be statistically significant.

At the same time, at almost all stages of observation, stimulated saliva was muddy, what is explained by the lack of proper nutrition and hygienic oral care. At the more distant stages of intensive training and clinical observation, we recorded more notable inhibition of salivary secretion. We suppose that at this stage the problem should be considered as a result of side effects physical activity, at the same time, the intake of special low-calorie food on the body, which is expressed in the tendency to increase functional disorders in the organs and tissues of the oral cavity, in particular, in marked inhibition of saliva secretion. During clinical studies of the periodontal tissues state and oral hygiene, as well as the degree of development of pathological processes in periodontium, the following criteria were applied: hygiene indices E. Quigley, I. Hein, 1962, Silness, Loe, 1967, and iodic number of Svrakov. Index data was recorded at several stages of the general training cycle: before the training process and immediately after it (table 2). The results were statistically processed and presented in the table below.

Table 2

Dynamics of changes in the values of dental indices in professional athletes

Indices	Group I (healthy periodontium), n = 57		Group II (with periodontal disease), n = 65	
	before training	1 month after training	before training	1 month after training
Quigley-Hein indices	2.14 ± 0.027	2.35 ± 0.023 $p < 0,001$	3.44 ± 0.019	4.19 ± 0.012 $p < 0,001$
Silness-Loe indices	1.36 ± 0.009	1.55 ± 0.008 $p < 0,001$	2.14 ± 0.020	2.75 ± 0.010 $p < 0,001$
iodic number of Svrakov	—	—	5.98 ± 0.034	6.52 ± 0.061 $p < 0,001$

Note: p - statistically significant differences in the parameters from groups before and after treatment ($p < 0.001$).

The hygienic condition of the oral cavity and the condition of soft periodontal tissues in the group of professional athletes with an intact periodontium worsened almost by 2 times after completion of intensive training ($p < 0.001$). A completely different picture and dynamics in the studied hygiene index was recorded in athletes who were diagnosed with chronic generalized periodontitis of mild severity, what the received index values testified to: for example, if in this group the values of the Quigley-Hein index at the beginning of training were an average of 3.44 ± 0.019 points, then after training completion they increased to the level of 4.19 ± 0.012 points, that is, increased almost 1.3 times ($p < 0.001$). A significant deterioration in the hygienic state of the oral cavity was observed against the background of excessive physical exertion, what was expressed in a decrease in the Silness-Loe index by almost 1.5 times ($p < 0.001$). Thus, during long and intensive trainings, was recorded defined strictly dynamics in a statistically significant increase in the hygiene and periodontal indices, as well as the iodic number of Svrakov.

In the group of athletes without any pathological changes in the periodontal tissues, the values of the Quigley-Hein hygiene index before the start of intensive training amounted to 2.14 ± 0.027 points, and Silness-Loe - 1.36 ± 0.009 points. After the completion of the basic preparatory part of the trainings and after the end of intense physical exertion, the obtained data differed in higher values and were determined respectively in the range of 2.35 ± 0.023 points and 1.55 ± 0.008 points ($p < 0.001$). Using for treatment and prevention of periodontal diseases and improving the functional state of the salivary glands, stimulating salivation of an alternative anti-inflammatory drug in the oral cavity, the examined athletes of all three groups were diagnosed with significant improvements in oral hygiene and the condition of periodontal tissues, as evidenced by the results of an index assessment and statistical analysis of the obtained results (table 3).

Based on the study of the obtained questionnaire data, it should be emphasized that at certain stages of training professional athletes, when, during the absence of special pre-competitive and competitive training cycles, they take relatively higher-calorie food and liquid, in very rare cases there were pronounced

deviations in the studied factors, indicating the functional state of organs and tissues of the oral cavity, including the salivary glands. After the use of a natural therapeutic and prophylactic agent based on propolis, the amount of excreted saliva and the rate of its secretion increased, but still they did not reach the values recorded in clinical studies of the oral cavity in the control group, which consisted of healthy people who did not play sports professionally.

Table 3

Changes in the condition of the oral cavity in professional athletes, before and after treatment

Observations Parameters	Before treatment		At 1 month after treatment	
	Group I (n=16)	Group II (n=15)	Group I (n=16)	Group II (n=15)
Silness-Loe indices	1.35±0.016	1.69±0.026	0.51±0.014 p<0,001	0.75±0.021 p<0,001
Bleeding index (Muhlemaim, 1971, Cowell I., 1975)	1.75±0.022	2.23±0.036	0.56±0.034 p<0,001	0.78±0.031 p<0,001
The speed of salivation, ml / min	1.56±0.018	2.24±0.036	1.81±0.029 p<0,001	2.43±0.029 p<0,001

Note: p - statistically significant differences in the parameters before and after treatment (p < 0.001).

Functional state of the salivary glands significantly changes both with a healthy periodontium and with the development of inflammatory processes in its soft tissues, as evidenced by the rate of background salivation, which underwent certain positive changes in all three observation groups. So, prior to the start of applications with a biologically neutral drug in the first group salivary flow rate was determined within the range of 2.24±0.036 ml / min, at the final stage of clinical trials, was observed a significant increase in rates up to 2.43±0.029 ml / min (p < 0.001). In professional athletes with chronic catarrhal gingivitis, the state of oral hygiene significantly improved, which was proved by the results of a statistical analysis of the hygiene indices data. At the same time, the digital values of the Silness-Loe index almost doubled and, if before the start of treatment, amounted to 0.51±0.014 points, then after the completion of therapeutic procedures using a biologically neutral drug, the indicators increased to 1.35±0.016 points, which indicated a significant improvement in the "environmental" situation in the oral cavity of qualified athletes.

As for the clinical assessment of periodontal soft tissues condition, in the main group was diagnosed a positive dynamics in the correction of pathological changes that were observed in the periodontal tissues against the background of intensive physical training, and that were manifested in a decrease in the values of the Mülleman-Cowell bleeding index.

A statistical analysis of the data of microbiological studies in oral cavity before and after the course of basic therapy revealed a decrease in the frequency of seeding of representatives of opportunistic and pathogenic microflora in biological samples taken a month after completion of treatment in both groups of examined qualified athletes (table 4). In the course of studies, microorganisms *Streptococcus haemolyticus*, *Staphylococcus aureus*, *Candida* were less common in the main group than in patients of the comparison control group.

Table 4

CFU of bacteria in the oral cavity of professional athletes before and after treatment

Microbial associations	(CFU)/ml			
	Group I Before treatment (n=16)	Group I 1 month after treatment (n=16)	Group II Before treatment (n=15)	Group II 1 month after treatment (n=15)
<i>Streptococcus haemolyticus</i>	1.07x10 ⁴ ±0.046	1.18x10 ² ± 0.037 (p<0.001)	1.18x10 ⁴ ±0.029	1.03x10 ⁴ ±0.060 (p<0.05)
<i>Staphylococcus aureus</i>	1.04x10 ⁵ ±0.065	1.11x10 ² ± 0.029 (p<0.001)	1.15x10 ⁵ ±0.071	0.92x10 ⁵ ±0.028 (p<0.01)
<i>Candida albicans</i>	1.17x10 ⁴ ±0.071	1.06x10 ² ± 0.042 (p<0.001)	1.04x10 ⁴ ±0.029	0.89x10 ⁴ ±0.050 (p<0.01)

Note: Significant differences between parameters before and after treatment (p < 0.05).

It should be noted a significant (p < 0.001) decrease in the number of the microbial associations which facilitates to the beginning and development of major dental diseases at the final stage of research after the use of natural preparations. After the complex treatment carried out using the Pomegranate Balm preparation, a more pronounced normalization of the microflora of the oral cavity contributed to a more pronounced improvement in the state of hygiene and periodontal tissues in athletes in the treatment of periodontitis. Some authors have compared the antimicrobial (antibacterial, antifungal and antiviral) activities and chemical composition of propolis of various origins. The results showed that, despite the large differences in the chemical composition of propolis from different geographic areas, all samples have

antibacterial, antifungal and antiviral effects. Studies have shown antibacterial activity against *Micrococcus luteus*, *Salmonella typhimurium*, *Klebsiella pneumoniae* [10, 15]. Some studies have demonstrated antimicrobial activity of propolis extract against gram-positive (*Staphylococcus aureus*, *Streptococcus piogenes*), gram-negative microorganisms (*Escherichia coli*, *Pseudomonas aeruginosa*) and yeast-like fungi (*Candida albicans*), as well as its antiviral activity in respiratory diseases [14]. Many authors have reported the sensitivity of some *Candida* yeasts, such as *Candida albicans*, to propolis [11, 12, 13].

As a result of a number of clinical and laboratory studies was determined the anti-inflammatory, antibacterial and immunomodulating efficacy of drugs based on propolis and its active biological components in the treatment and prevention of various dental diseases. Experimental studies of propolis extracts have shown that they improve the condition of the gums and periodontium [9].

Conclusions

One of the achievements of these studies is the fact of widespread introduction propolis-based preparations in modern practical dentistry, due to the presence in its composition of biologically active macro- and microelements, especially for preventive purposes, for increased work ability, body defenses and stress resistance of professional athletes, for improving their quality of life and sports performance.

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Реферати

ОПТИМІЗАЦІЯ ЗАХОДІВ КОРЕКЦІЇ СТОМАТОЛОГІЧНОГО ЗДОРОВ'Я У ПРОФЕСІЙНИХ СПОРТСМЕНІВ Сафаралиєв Ф.Р.

У 122 професійних спортсменів-борців у віці від 18 до 34 років було вивчено клінічний стан порожнини рота, функціональний стан слинних залоз і ефективність лікувально-профілактичних заходів з використанням ліків на основі прополісу (Гранатовий бальзам). В ході наших спостережень було констатовано факт значного і несприятливого зниження фонові нестимульованій секреції слини у обстежуваних спортсменів-диноборців в досліджувані терміни перебування в тривалому напруженому психоемоційному та фізичному стані. Стан гігієни порожнини рота і

ОПТИМІЗАЦІЯ МЕР ПО КОРЕКЦІЇ СТОМАТОЛОГІЧЕСКОГО ЗДОРОВ'Я У ПРОФЕСІОНАЛЬНИХ СПОРТСМЕНОВ Сафаралиєв Ф.Р.

У 122 професійних спортсмена-борця в віці від 18 до 34 років були вивчені клінічне стан порожнини рота, функціональне стан слинних залоз і ефективність лікувально-профілактичних заходів з використанням лікарства на основі прополіса (Гранатовий бальзам). В ході наших спостережень був констатований факт значного і несприятливого зниження фонові нестимульованій секреції слини у обстежуваних спортсменів-диноборців в досліджувані терміни перебування в тривалому напруженому психоемоційному та фізичному стані. Стан гігієни порожнини рота і

околозубних тканин в досліджуваній групі спортсменів, що мають практично здоровий пародонт, погіршилися після закінчення інтенсивного тренувального процесу майже у 1,5 рази. Препарат «Бальзам гранатовий» володіє дуже важливими, особливо з профілактичної точки зору, протизапальними, органолептичними властивостями, які на підставі природної стимуляції сприяють поліпшенню гігієнічного стану і мікрофлори порожнини рота, стану тканин пародонта, а також вираженій стимуляції слиновиділення і підвищенню швидкості слиновиділення.

Ключові слова: пародонт, слина, гігієна, фізичні навантаження, профілактика.

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околозубных тканей в исследуемой группе спортсменов, имеющих практически здоровый пародонт, ухудшились по окончании интенсивного тренировочного процесса почти в 1,5 раза. Препарат «Бальзам гранатовый» обладает очень важными, особенно с профилактической точки зрения, противовоспалительными, органолептическими свойствами, так как на основании естественной стимуляции способствуют улучшению гигиенического состояния и микрофлоры полости рта, состояния тканей пародонта, а также выраженной стимуляции слюноотделения и повышению скорости слюноотделения.

Ключевые слова: пародонт, слюна, гигиена, физические нагрузки, профилактика.

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DETERMINATION OF THE RISKS OF INFERTILITY IN WOMEN WITH THYROID PATHOLOGY AND HYPOANDROGENIC OVARIAN DYSFUNCTION

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The purpose of the study was to determine the effect of thyroperoxidase antibody levels on the fertility of women with sexual dysfunction and reduced ovarian androgen levels. A study of the risks of infertility in women with pathology of the thyroid gland based on the analysis of the results of hormonal tests and the development of recommendations for planning a pregnancy in conditions of hypoandrogenic ovarian dysfunction. The presence of clinically significant levels of antibodies to thyroperoxidase in young and middle-aged women with sexual dysfunction has been shown to adversely affect the early stages of folliculogenesis. This factor indicates a risk of decreased steroid-producing ovaries and the development of susceptibility to infertility. It is desirable for women with clinically significant levels of antibodies to thyroperoxidase to plan their pregnancy at an earlier reproductive age (18-25 years), because at this time the optimal conditions for fertility are maintained. For women with clinically low levels of antibodies to thyroperoxidase, pregnancy planning is more appropriate in middle reproductive age.

Keywords: infertility, antibodies, androgen deficiency.

The work is a fragment of the research project "Reproductive Health and Sexual Dysfunction of Women of Reproductive Age with Androgen Deficiency. Development of diagnostic criteria", state registration No. 0119U001422.

The quality of life of a person depends on the realization of its reproductive and sexual function. There are studies of the negative effects of hyperandrogenism on female fertility [11]. Much attention is paid to the study of the sexual health of women, since a woman's sexual health is part of her reproductive health [10].

The endocrine and nervous systems regulate the main physiological processes that ensure sexual function of a person. The neuroendocrine system is the basis of female sexuality: it provides excitement of the relevant nervous structures responsible for sexual reactions, supports the energy component of sexual desire, sexual motivation (sexual centers of the hypothalamus, which affect the sexual centers of the spinal cord, which are subject to the regulatory effects of the limbic system and the cerebral cortex) [2, 11].

Reduced women sexuality is noted against the backdrop of various endocrine diseases as they alter the levels of estrogen and progesterone in the female body. One of the most common diseases of reproductive age women is autoimmune thyroiditis (AIT), an organ-specific autoimmune disease of the thyroid gland, which is the main cause of hypothyroidism. The value of the hypothyroid condition for fertility is due to the high frequency of this pathology - up to 78.4% in women with infertility [1, 9]. It is difficult to estimate the prevalence of AIT, since in the state of euthyroidism it has almost no exact diagnostic criteria. Many authors point out that the number of carriers of both antibodies to thyroid peroxidase and hypothyroidism due to autoimmune thyroiditis is about 10 times higher among women in comparison with men [5]. Quite often, the hypothyroid state is accompanied by hyperprolactinemia. According to the article for women with hyperprolactinemia there is an increase in the frequency of depressive disorders and disorders of the menstrual cycle in combination with a decrease in sexual desire,

lubrication, difficulty in arousal, orgasmic dysfunctions [8]. Therefore, sexual disorders were diagnosed according to the DSM-5 classification [12].

The purpose of the work was to determine the most favorable time for fertility in women with autoimmune thyroid disease and low ovarian androgens.

Materials and methods. The total number of surveyed women of different reproductive ages (18 to 35 years) is 80 people. They all had complaints of a decrease in sexual desire and discomfort during sexual intercourse. All women applied to the Reproductive Medicine and Surgery department of the Ukrainian scientific and practical center of endocrine surgery, transplantation of endocrine organs and tissues of the Ministry of Health of Ukraine. All women had no pregnancy history and planned a gestational debut. The clinical manifestation of sexual dysfunction was more than 6 months.

The study did not include patients after thyroid surgery; after radioiodine therapy; patients receiving amiodarone or glucocorticoids, as these drugs may affect thyroid functional activity.

Patients underwent clinical and laboratory examination at the center's biochemical laboratory. According to the results, women were divided into two age groups: 18-25 years – early reproductive age (I-a, I-b), and 25-35 years – average reproductive age (II-a, II-b). The I-a and II-a groups included women with clinically insignificant indicators of antibodies to thyroperoxidase, and to I-b, and II-b – with clinically significant indicators of antibodies to thyroperoxidase. Thyroperoxidase antibodies were considered to be clinically insignificant up to 35 IU/ml. All patients were in a state of euthyroidism (TSH did not exceed $4.0\mu\text{ME/ml}$). Determination of hormonal homeostasis of the examined women was performed in the first phase of the menstrual cycle (5-7 days), which is the main indicator of the onset of ovarian steroidogenesis. It is associated with follicle growth.

Statistical data processing was performed using the STATISTICA for WINDOWS software package (version 5.5). For the results obtained, methods were used to evaluate the law of distribution of the obtained data both for a separate group and for the population as a whole. For values with a normal distribution, average values were used together with the standard deviation as the main characteristics of the studied values. To analyze the dependencies, the parameters of the regression equation for the sample were estimated. When analyzing the existence of significant differences for the two groups, the Student criterion was used.

Results of the study and their discussion. Analysis of the sexual function of women revealed the following: the frequency of sexual intercourse per week for women of I-a and II-a groups practically did not change with age 3.9 ± 1.04 and 4.10 ± 1.12 , while for women I-b and II-b, there was a decrease in sexual activity from 3.25 ± 1.03 to 2.5 ± 0.70 times a week.

According to the main indices of hormonal support the reference norms for follicle-stimulating hormone (FSH) indicator are set at 2.8-1.3 IU/l. For the women of I-a and II-a groups the follicle-stimulating hormone (FSH) index was 4.2 (3.65-4.79) IU/l and 5.35 (4.89-5.81) IU/l (growth by 1.3 times). For women in groups I-b – 4.70 (3.94-5.46) IU/l and II-b – 7.60 (6.39-8.81) IU/l (increase in 1,6 times).

The LH level for women in group I-a was 4.27 (4.00-4.54) IU/l, and for women in group II-a was 5.12 (4.88-5.36) IU/l that is, a slight increase with age (reference rates 1.9-12.5 IU/l). LH index for women in b-group (I-b – 3.69 (3.19-4.20) IU/l and II-b – 4.14 (3.43-4.84) IU/l) has the same a tendency to a slight increase with age.

In this case, the FSH/LH index for group I-a is 0.99, and for group I-b – 1.04, that is, it has practically not changed and complies with the norm for the beginning of follicles maturation. For women of groups II-a (1.27) and II-b (1.9) this ratio tends to increase.

In reproductology, an LH/FSH ratio of less than 0.5 consider as negative for follicle maturation. The LH/FSH index for women of group I-a it was 1.04, for women of group II-a it was 0.96; for women of group I-b it was 0.8, for women of group II-b it was 0.56 .

This indicates that peptide hormone synthesis rates for women with clinically relevant levels are insufficient to adequately synthesize ovarian steroids already at the onset of follicular growth.

For women of group I-a, the level of estradiol was 47.38 (37.74-57.02) pg/l, in group II-a it was 73.14 (82.68- 63.61) pg/l, which shows an increase by 1.5 times (reference norms 19.5-144.2 pg/l). For women of group I-b this index made 67.45 (78.20-56.70) pg/l, and for women of group II-b it made 54.70 (46.44-62.96) pg/l, which characterizes a decrease of the index value with age by 1.23 times. This indicates an opposite tendency in comparison to women of I-a and II-a groups . Analysis of progesterone for women in group I-a determined the level of 0.45 (0.34-0.56), for women in group II-a it was 0.75 (0.64-0.87) ng/ml (increase by 1.66 times). A similar index for women of group I-b was 0.71 (0.46-0.97) ng/ml, and for women of group II-b it was 0.26 (0.22-0.30) ng/ml (decrease by 2.7 times) (reference norms 0.2-1.4 ng/ml).

This means that, with age, progesterone levels for women with clinically significant AT-TPO levels tended to have a significant trend (the probability, that the mean progesterone values for these age groups were obtained from general groups having the same meaning, is less than 0.005) reduction, although it remained within the reference rate for first phase of the cycle.

Androgens are precursors of estrogens in their biosynthesis process. They are extremely important for the reproductive function and maintenance of hormonal women homeostasis at different ages. Androgen biosynthesis is carried out in the cells of preantral and antral follicles under the influence of LH and is provided by six enzyme systems that are necessary for the conversion of cholesterol into testosterone. We analyzed the main androgens of women: total testosterone (Ttot) and DHEA-S.

For women of group I-a Ttot was 0.92 (0.78-1.05) nmol/l, for women of group II-a Ttot was 0.67 (0.52-0.83) nmol/l. These show a decrease by 1.3 times with age (reference rate 0.38-1.97 nmol/l). For women of group I-b the level of Ttot was 0.65 (0.50- 0.79) nmol/l, for women of II-b it was 0.41 (0.38-0.44) nmol/l. The decrease with age ranks up to 1.58 times. It means common testosterone for women with sexual dysfunction and decreased lubrication was a decrease in levels with age. The rate of decline is greater for women with clinically significant levels of antibodies to thyroperoxidase.

The reference norms for DHEA-S depend on age. For 15-20 years old women it is 88-483 mcg/dl, for 20-30 years old women it is 280-640 mcg/dl, for 30-40 years old women it is 120-520 mcg/dl. DHEA-S for women of group I-a it was 154.95 (146.56-163.34) mcg/dl, for women of group II-a it was 134.78 (112.13-157.42) mcg/dl (decrease by 1.14 times). For women of group I-b this index was equal to 102.79 (80.15-125.44) mcg/dl, and for women of group II-b group this index value was equal to 59.83 (52.67-66.94) mcg/dl (decrease by 1.7 times).

This suggests that a decrease in testosterone and dehydroepiandrosterone sulfate levels with age was common for women with sexual dysfunction and decreased lubrication. This decrease is more significant for women with clinically significant levels of antibodies to thyroperoxidase .

For women of group I-a, the cortisol index was 22.35 (20.27-24.42) mg/dl, for women of group II-a, the cortisol level was 15.57 (13.93-17.21) mg/dl, showing a 1.4 – fold decrease (benchmark being 4.30-22.40 mg/dl). For women of group I-b this index value was at the level of 20.19 (17.30-23.07) mg/dl, and for women of group II-b it reached 22.22 (20.64-23.79) mg/dl. This suggests that with age there is a tendency to approach the level of cortisol to the upper limit of normal.

The analysis of prolactin level showed the following: for women of group I-a the prolactin ratio reached 16.06 (13.95-18.16) ng/ml, for women of group II the index value was 15.71 (13.58-17.85) ng/ml, (reference values of 2.8-29.2 ng/ml). For women in group I-b prolactin was 14.82 (13.48-16.16) ng/ml, and for women in group II-b it was 13.17 (9.48-16.85) ng/ml.

This indicates that no hyperprolactinemia was observed in both groups of women surveyed.

This means that, with age, progesterone levels for women with clinically significant levels of antibodies to thyroperoxidase. Levels of antibodies to thyroperoxidase tended to have a significant trend (the probability, that the mean progesterone values for these age groups were obtained from general groups having the same meaning, is less than 0.005) to reduction, although it remained within the reference rate for first phase of the cycle.

Analysis of cortisol levels for women with ovarian dysfunction and androgen deficiency indicates high levels of stress in women of early reproductive age, regardless of the level of antibodies to thyroperoxidase. With age, cortisol levels remain high for women with clinically significant levels of antibodies to thyroperoxidase. For women without high rates of antibodies to thyroperoxidase, cortisol levels come to mean reference rates.

Our observations are consistent with the findings of other researchers [4, 7], who note the initial increase in the concentration of LH and FSH in stress states. Such growth is likely due to the stimulating effect of the corticotropin-releasing hormone. Sexual steroid hormones play a very important role in the system's normal functioning of the body as a whole. For women with clinically relevant levels of antibodies to thyroperoxidase and sexual dysfunction during their lifetime, we have found an increase in FSH/LH imbalance at the dominant follicle sampling stage. This condition is accompanied by significantly lower ($p < 0.05$) estradiol values and progesterone insufficiency in the first phase of the menstrual cycle.

Testosterone helps maintain an adequate response of the body to stress. Duration of stressor is a determining factor for testosterone synthesis [6]. For women with sexual dysfunction, our findings also indicate a decrease in testosterone levels. This decrease is more pronounced for women with clinically relevant rates of antibodies to thyroperoxidase. We found a significant decrease in the level of dehydroepiandrosterone sulfate (DHEA-S) for women with thyroid pathology and sexual dysfunction, indicating an intracrine negative effect of this hormone deficiency on estradiol secretion and steroid

deficiency. The authors, who studied the value of DHEA-S for successful generative function of women, noted that it is the normalization of the DHEA-S level and leads to an increase in the concentration of insulin-like growth factor-1 (ILGF-1) of follicular origin, which indirectly stimulates the influence of gonadotropin and suppresses the process of their premature atresia [3].

Conclusion

1. The presence of clinically relevant levels of antibodies to thyroperoxidase for women of early and middle age with sexual dysfunction adversely affects the early stages of folliculogenesis. This factor indicates the risk of decreased steroid-producing ovarian function and the formation of a tendency for their androgen deficiency.

2. It is advisable for women with clinically relevant levels of antibodies to thyroperoxidase to plan their pregnancy at an earlier reproductive age (18-25 years), since optimal conditions for fertility are maintained at this time. With age, such women have the conditions for inadequate synthesis of ovarian steroids at the beginning of follicular growth, which increases the risk of primary infertility.

3. It is more appropriate for women with sexual dysfunction and clinically insignificant levels of antibodies to thyroperoxidase to plan for pregnancy in the middle reproductive age. This reduces the risk of primary infertility too.

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Реферати

ВИЗНАЧЕННЯ РИЗИКІВ ФОРМУВАННЯ БЕЗПЛІДДЯ У ЖІНОК З ПАТОЛОГІЄЮ ЩИТОВИДНОЇ ЗАЛОЗИ І ГІПОАНДРОГЕНОВОЮ ДИСФУНКЦІЄЮ ЯЄЧНИКІВ

Семенюк Л.М., Юзвенко Т.Ю., Бородкін Г.О., Крижановська О.І.

Метою роботи було визначення впливу рівня антитіл до тиреопероксидази на фертильність жінок із сексуальною дисфункцією, та зниженням рівнем яєчникових андрогенів. Проведено дослідження ризиків формування безпліддя у жінок з патологією щитовидної залози на основі аналізу результатів гормональних тестів та розробки рекомендацій щодо планування вагітності в умовах гіпоандрогенної дисфункції яєчників. Встановлено, що наявність клінічно значущих рівнів антитіл до тиреопероксидази у жінок раннього та середнього віку із статевою дисфункцією негативно впливає на ранні стадії фолікулогенезу. Цей фактор вказує на ризик зниження функціонування яєчників, що продукують стероїди, та формування сприйнятливості до безпліддя. Бажано жінкам з клінічно значущим

ИССЛЕДОВАНИЕ ФОРМИРОВАНИЯ БЕСПЛОДИЯ У ЖЕНЩИН С ПАТОЛОГИЕЙ ЩИТОВИДНОЙ ЖЕЛЕЗЫ И ГИПОАНДРОГЕНОВОЙ ДИСФУНКЦИЕЙ ЯИЧНИКОВ

Семенюк Л.Н., Юзвенко Т.Ю., Бородкин Г.А., Крыжановская О.И.

Целью работы было определение влияния уровня антител к тиреопероксидазе на фертильность женщин с сексуальной дисфункцией и сниженным уровнем яичниковых андрогенов. Проведено исследование рисков формирования бесплодия у женщин с патологией щитовидной железы на основе анализа результатов гормональных тестов и разработки рекомендаций по планированию беременности в условиях гипоандрогенной дисфункции яичников. Установлено, что наличие клинически значимых уровней антител к тиреопероксидазе у женщин раннего и среднего возраста с половой дисфункцией негативно влияет на ранние стадии фолликулогенеза. Этот фактор указывает на риск снижения функционирования яичников, продуцирующих стероиды и формирование восприимчивости к бесплодию. Желательно женщинам с клинически значимым уровнем

рівнем антитіл до тиреопероксидази планувати свою вагітність у більш ранньому репродуктивному віці (18-25 років), оскільки в цей час підтримуються оптимальні умови для народжуваності. Для жінок з клінічно незначним рівнем антитіл до тиреопероксидази планування вагітності є більш доцільним у середньому репродуктивному віці.

Ключові слова: безпліддя, антитіла, андрогенна недостатність.

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антител к тиреопероксидазе планировать свою беременность в более раннем репродуктивном возрасте (18-25 лет), поскольку в это время поддерживаются оптимальные условия для рождаемости. Для женщин с клинически незначительным уровнем антител к тиреопероксидазе планирования беременности является более целесообразным в среднем репродуктивном возрасте.

Ключевые слова: бесплодие, антитела, андрогенная недостаточность.

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RATIONALE FOR THE USE OF METHODS OF MICROSCOPIC CRYSTALLOGRAPHY IN FRACTALS OF PROTEINS AND IRRIGATION FOR THE EARLY DIAGNOSIS, TREATMENT AND PREVENTION OF INFLAMMATORY PROCESSES IN THE ORAL CAVITY

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In dentistry, new methods of prevention and treatment of diseases caused by aggressive biofilm are constantly offered. If timely oral hygiene and preventive measures are ignored or underestimated, the impact of aggressive biofilm can be irreversible and cause changes in periodontal tissues. One of the key factors that worsen the general state of oral health is the presence of removable and fixed dentures in patients. This is because soft dental plaque forms much faster on the surface of implant. Today, one of the most effective and optimal methods used for the prevention and treatment of inflammatory processes in the oral cavity is irrigation. We examined a total of 90 people. The age of the patients ranged from 40–60 years. Of these, there were 40 men and 50 women. Analyzing the value of the protein fractals, namely the area, we assessed and confirmed the hygienic condition of the oral cavity in people with dental prostheses. An application of the periodontal tissue irrigation method for fixed dental prostheses leads to a decrease in the percentage of protein fractals area in the digital crystallography samples. The results are confirmed by statistical and clinical data.

Key words: microscopic crystallography, irrigation, oral cavity, periodontium, metal-ceramic fixed dental prostheses.

The work is a fragment of the research project "Restoration of dental health in patients with major diseases and their rehabilitation", state registration No. 0116U004191.

Hygienic condition of the oral cavity directly affects the development of periodontal disease. One of the leading negative factors is dental plaque. At the initial stage, it contains aerobic microorganisms that demineralize the enamel. The mature dental plaque is dominated by anaerobic bacteria associated with the etiology of gingivitis and periodontitis. Such bacteria penetrate into the gingival pockets, secrete toxins and enzymes that lead to the destruction of periodontal tissues [1].

In dentistry, new methods of prevention and treatment of diseases caused by aggressive biofilm are constantly offered. If timely oral hygiene and preventive measures are ignored or underestimated, the impact of aggressive biofilm can be irreversible and cause changes in periodontal tissues. One of the key factors that worsen the general state of oral health is the presence of removable and fixed dentures in patients. This is because soft dental plaque forms much faster on the surface of implant [5, 6].

Poor oral hygiene significantly reduces the life of dentures, and subsequently leads to periodontal disease.

The study of biofilms formed in the natural habitat revealed significant differences between planktonic and biofilm forms of microbiota, including differences in bacterial behavior, biochemical processes, biosynthesis of various products, information exchange, including genetic one. Obviously, biofilms can promote the transfer of resistance genes to antibiotics and to various chemical biocides. As a result of inflammation initiated by biofilm microbes, the pH in the gingival sulcus increases and can accelerate the growth and proteinase activity of some periodontal pathogens by activating the processes of gene expression [2, 3, 5].

This helps to understand why systemic antimicrobials that are applied topically are not always effective, even in cases where the antibacterial drug is selected by the spectrum of its action and activity on a particular type of periodontal pathogens. The latter allows us to explain why mechanical dental plaque removal and hygienic measures (personal and professional hygiene) are the most important component of comprehensive treatment of patients with periodontal disease in practical dentistry.

Taking into account the data of domestic and foreign sources for application of fixed dentures, namely porcelain-fused-to-metal crowns, has a connection with the risk of activation of pathogenic mechanisms which cause in the periodontal tissues localized, and with timely diagnosis, an inflammatory process, which can be treated in the initial stages of detection [4].

According to the literature, to date, one of the effective and optimal methods used for the prevention and treatment of inflammatory processes in the oral cavity is irrigation [7, 8].

The positive irrigation effect on periodontal tissues is explained by the fact that the specific interaction of the organism and microbes is disturbed, which is what leads to the elimination of signs of inflammation in the oral tissues.

The main effect of modern irrigators is the ability to mechanically eliminate aggressive biofilm, microbial cells and food particles by washing it all down with a regulated water flow, and further resist its formation.

Timely diagnosis of periodontal diseases, full assessment of treatment results in the near and long-term periods, and most importantly – preventive measures to ensure good oral hygiene are becoming an urgent problem in modern dentistry.

The purpose of the study was to rationale for the use of methods of microscopic crystallography in fractals of proteins and irrigation method for the early diagnosis, treatment and prevention of inflammatory processes in the oral cavity.

Materials and methods. Patients were divided into groups according to the presence of fixed dental prostheses, namely porcelain-fused-to-metal ones. We examined a total of 90 people. The age of the patients ranged from 40–60 years. Of these, there were 40 men and 50 women.

All patients applied to the UMSA Department of Postgraduate Education of Dentists and to the Orthopedic Department of the Scientific-Educational-Medical Dental Center where they underwent a full preventive examination and further treatment.

The criteria for the distribution of the studied patients were: the term of use of dental prostheses, the presence of dentition defects and periodontitis of I-II degree.

The group formation was as follows: Group number 1 – numbered 29 people. The term of dentures using was less than 4 years. The second group included persons in whom dental prostheses were used for more than 4 years.

Crystallographic image of rinsing from the oral cavity in patients with fixed dental prostheses (fig. 1).



Fig. 1. Samples of protein fractals in the crystallographic image of oral fluid.

Statistical data processing was performed using the Satus sETT 10 software package tool.

Analyzing the value of the protein fractals, namely the area, we assessed and confirmed the hygienic condition of the oral cavity in people with dental prostheses.

The oral cavity and metal-ceramic fixed dentures irrigation method was performed using an "ACleon TF600" irrigator. Irrigation was carried out by a course of 0.05% aqueous solution of chlorhexidine bigluconate, it consisted of 2 sessions carried out over an interval of three weeks.

The ACleon TF600 irrigator contains seven nozzles, including a periodontal one, which has a pointed base and a thin tip made of

special rubber, which allowed the liquid to get into the gingival sulcus and periodontal pocket. An orthopedic nozzle was used for mechanical cleaning of the fixed dentures. This procedure lasted, on average, 4–6 minutes.

The need for treatment of patients who used dental prostheses and further control of crystallographic samples of digital processing was determined by the CPITN index.

Factors of assessment were: gingival bleeding, namely its presence or absence; signs of subgingival and supragingival calculus; gum pockets – shallow (4-5 mm) and deep (6 mm or more). Another dental plaque index was the Silness – Loe index. Statistical data were processed using a licensed package (STATISTIK 10.0).

The data of the dependent variables were used to estimate the numerical indices of digital occlusiography and cone-beam tomography. In the case when the index was $p > 0.05$, the use of parametric analysis of variance was rationale, according to $p < 0.05$, we used non-parametric analysis of variance.

The correlation force (correlation coefficient – r) was interpreted as follows: $r \leq 0.25$ – weak correlation; $0.25 < r < 0.75$ – moderate correlation; $r \geq 0.75$ – strong correlation.

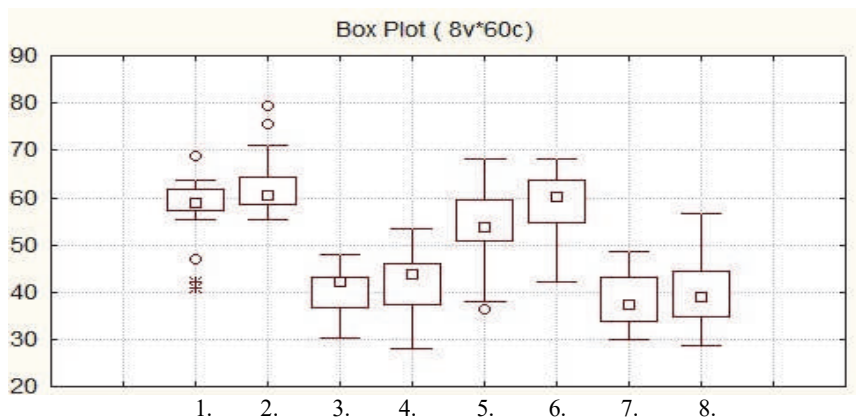


Fig. 2 Graph of fluctuations in the values of the protein fractal areas. Images of the studied groups at the different stages of treatment. 1.Group I (before the first irrigation); 2.Group II (before the first irrigation); 3.Group I (after the first irrigation); 4.Group II (after the first irrigation); 5.Group I (before the repeated irrigation); 6.Group II (before the repeated irrigation); 7.Group I (after the repeated irrigation); 8.Group II (after the repeated irrigation)

Results of the study and their discussion. Simultaneous evaluation of the values of the first and second experimental groups before and after the use of the irrigation method proves the influence of this method on the tesigraphic picture, which confirms the decrease (in percent) of PFA (protein fractal area) to the total area of the figure, and this is confirmed according to this parametric analysis (fig. 2).

Table 1

Table of data of correlation multifactor analysis in the protein fractal areas of oral fluid according to digital morphometrics of a tesigraphic sample Multifactor Variance Correlation Analysis

Effect	Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	7582.53	1	7582.531	306.1211	0.000000
A	9.57	1	9.566	0.3862	0.542550
B	11724.73	2	5862.365	236.6748	0.000000
C	294.21	3	98.071	3.9593	0.026090
Error	421.08	17	24.770		

Thus, correlation analysis shows the dependence of indices on each other. Namely: A – index of using fixed dental prostheses in patients of the experimental group (< and > 4 years). Factor B as an index of the data of the applied methods of oral hygiene in patients who used metal-ceramic fixed dental prostheses. And the last factor, also known as index C, which characterizes the use of methods for determining hygiene (table 1).

This correlation of factors showed the high quality of the correlation model verification data (table 2).

Table 2

Correlation factor verification data table

Dependent Variable	Multiple R	R ²	Adjusted R ²	SS Model	df Model	MS Model	SS Residual	df Residual	MS Residual	F	p
Аналіз	0.982943	0.966177	0.954239	12028.51	6	2004.751	421.0850	17	24.76971	80.93561	0.000000

The obtained results of numerical values of the CPITN index in the first group before treatment gave the following results: the patients who did not need treatment were 0 people. Provided recommendations on improvement of hygienic care of the oral cavity – two persons. Five people needed professional hygiene. Topical anti-inflammatory therapy was clearly recommended for a group of 19 patients. Four patients required comprehensive treatment of the entire oral cavity (fig. 3a).

Data from the CPITN index showed the following results of the treatment: 15 patients were recommended to improve their oral hygiene. It was important to reduce the number of subjects who were shown topical anti-inflammatory therapy. There were 4 people. In other words, their number decreased from 19 to 4 people. And the number of patients who were recommended for professional hygiene decreased by 1 person. An important factor was that the number of people who were recommended complex therapy reduced to 0 (fig. 3b).

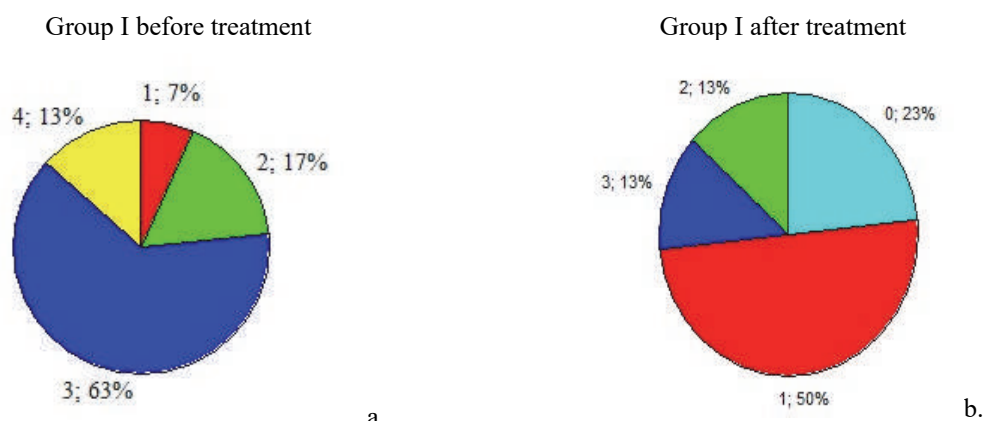


Fig. 3 (a, b). Image of the CPITN index chart in the first group before treatment (a) and after treatment (b). 0 – treatment is not required; 1 – patient should improve hygiene; 2 – patient requires a course of professional hygiene; 3 – was indicated topical anti-inflammatory therapy; 4 – was indicated a comprehensive treatment

In Group II there were no patients with absolute well-being before treatment, and after it, their number in this group increased to three people. The distribution of patients by other categories in group II was as follows: recommended to improve oral hygiene – one patient; required professional hygienic intervention – 4 people; topical anti-inflammatory therapy was indicated for 20 patients; recommended comprehensive treatment of periodontal tissues – 5 patients (fig. 4a).

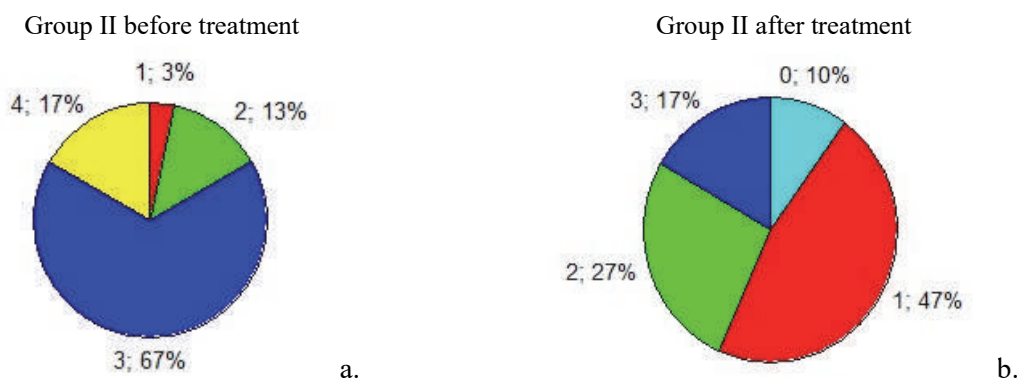


Fig. 4 (a, b). Image of the CPITN index chart in the second group before treatment (a) and after treatment (b). 0 – treatment is not required; 1 – patient should improve hygiene; 2 – patient requires a course of professional hygiene; 3 – was indicated topical anti-inflammatory therapy; 4 – was indicated a comprehensive treatment

According to the results of the treatment: therapeutic intervention was not indicated for three people; improvement of hygienic care of the oral cavity amounted to – 14 patients; professional hygienic intervention was shown to 8 patients, and anti-inflammatory therapy, decreased from 20 to 5 people; the treatment resulted in the absence of a category of patients who were shown a comprehensive treatment (fig. 4b).

The issue of quality diagnosis of the hygienic condition of the oral cavity is paid more and more attention every year. Thus, Mira A., Simon-Soro A., Curtis M. A. Role noted in their studies the importance and prospects of modern digital methods for estimating the area of protein fractals in the digital crystallographic image of oral fluid.

It is important that the value of this index at the stage of initial control differed in the studied groups – in the first group the index of protein fractal area was 55.158%, and in the second group – 62.35%. Thus, in subjects who used fixed dental prostheses for more than 4 years, PFA was higher by 7.10%. Thus, the PFA index increases with the period of using metal-ceramic fixed dental prostheses.

Nazarchuk O. A., Faustova M. O. [5] in their works studied the effect of the mechanism of irrigation on the of oral tissues, explaining the violation of the specificity of the interaction of the organism and microbes, which causes a decrease in the signs of the oral cavity inflammation. The authors Ippolotov Ye. V., Nikolaeva Ye. N., Tsarev V. N. [3] gave an important role to the study of aggressive biofilm as inducers of innate immune signaling systems, and Orekhova L.Yu., Zhavoronkova M. D., Suborova T. N [6] emphasized the creation of modern methods for diagnosing the hygienic status of the patient. In our study, the estimation of dynamic changes in digital microscopic crystallography was clinically confirmed, namely by the methods of index CPITN estimation and Silness – Loe and confirmed by the data of Dubina V.O., Skrypnykov P.M., Khavalkina L.M. and others [2] on the influence of the irrigation method on the course of inflammatory processes in periodontal tissues.

One of the important proofs of our proposed method of irrigation and evaluation of protein fractals was multifactor correlation analysis of variance. It allowed to prove, firstly, the informativeness and clinical effectiveness of the above method of determining the hygienic condition of the oral cavity in comparison with well-known methods of index assessment, which was confirmed by estimating the high level of this correlation model ($R = 0.983$; $R^2 = 0.966$).

Conclusion

The use of periodontal tissue irrigation method for fixed dental prostheses leads to a decrease in the percentage of PFA digital crystallography samples according to: from 55.158% to 37.874% in Group I, from 62.35% to 39.407% in the second experimental group. It remains important that according to digital crystallography, the second group had more patients at all stages of treatment.

Clinical confirmation was based on changes in microscopic crystallography by CPITN and Silness – Loe indices, as the treatment resulted in a decrease in the mean CPITN from 2,933 to 1,266 in Group I and from 3,066 to 1,612 in Group II. The same changes occurred when determining the Silness – Loe index, it decreased from 3,266 to 1,253 in Group I and from 3,633 to 1,421 in Group II.

After interpreting the clinical assessment according to the CPITN indices, it was determined that there were dynamic changes and as a result of the treatment we received the following data: the category of patients who showed only improvement of self-hygiene increased from 7% to 50% in the first group and from 3% to 47% in the second group, the following data showed a decrease in the number of people recommended topical anti-inflammatory therapy from 63% to 13% in the first group and from 67% to 17% in the second group.

According to the analysis of variance of multifactor indices where $R = 0.983$, $R^2 = 0.966$, the validity of the values of digital crystallography of microscopic samples as an important indicator of the level of hygienic condition of the oral cavity was proved.

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Реферати

ОБГРУНТУВАННЯ ВИКОРИСТАННЯ МЕТОДІВ МІКРОСКОПІЧНОЇ КРИСТАЛОГРАФІЇ БІЛКОВИХ ФРАКТАЛІВ ТА ІРИГАЦІЇ ДЛЯ РАННЬОЇ ДІАГНОСТИКИ, ЛІКУВАННЯ ТА ПРОФІЛАКТИКИ ЗАПАЛЬНИХ ПРОЦЕСІВ ПОРОЖНИНИ РОТА

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В стоматології постійно пропонують нові методи профілактики та лікування захворювань викликаних агресивною біоплівкою. Якщо, ігнорувати чи недооцінювати своєчасну гігієну порожнини рота та профілактичні заходи, вплив агресивної біоплівки може бути незворотнім і викликати зміни в тканинах пародонту. Одним із головних факторів які погіршують загальний стан гігієни порожнини рота є наявність знімних та незнімних протезів у пацієнтів. Це зумовлено тим, що на поверхні штучних зубів м'який зубний наліт формується набагато швидше. На сьогоднішній день одним із дієвих і оптимальних методів, який застосовують для профілактики і лікування запальних процесів порожнини

ОБОСНОВАНИЕ ИСПОЛЬЗОВАНИЯ МЕТОДОВ МИКРОСКОПИЧЕСКОЙ КРИСТАЛЛОГРАФИИ БЕЛКОВЫХ ФРАКТАЛОВ И ИРРИГАЦИИ ДЛЯ РАННЕЙ ДИАГНОСТИКИ, ЛЕЧЕНИЯ И ПРОФИЛАКТИКИ ВОСПАЛИТЕЛЬНЫХ ПРОЦЕССОВ ПОЛОСТИ РТА

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В стоматологии постоянно предлагают новые методы профилактики и лечения заболеваний вызванных агрессивной биопленкой. Если, игнорировать или недооценивать своевременную гигиену полости рта и профилактические мероприятия, влияние агрессивной биопленки может быть необратимым и вызвать изменения в тканях пародонта. Одним из главных факторов ухудшающих общее состояние гигиены полости рта является наличие съемных и несъемных протезов у пациентов. Это обусловлено тем, что на поверхности искусственных зубов мягкий зубной налет формируется гораздо быстрее. На сегодняшний день одним из действенных и оптимальных методов, применяемых для профилактики и лечения воспалительных процессов полости рта является

рота є іригація. Нами було обстежено загалом 90 осіб. Вік пацієнтів варіював від 40-60 років. З них 40 чоловік та 50 жінок. Аналізуючи значення показника білкових фракталів, а саме площа, ми оцінили та підтвердили гігієнічний стан ротової порожнини у осіб які використовували ортопедичні конструкції. Застосування методу іригації тканин пародонта для незнімних ортопедичних конструкцій призводить до зменшення відсоткової частини площі білкових фракталів цифрових зразків кристаллографії. Результати підтверджені статистичними і клінічними даними.

Ключові слова: мікроскопічна кристаллографія, іригація, порожнина рота, пародонт, металокерамічні незнімні ортопедичні конструкції.

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ирригация. Нами было обследовано 90 человек. Возраст пациентов варьировал от 40-60 лет. Из них 40 мужчин и 50 женщин. Анализируя значение показателя белковых фракталов, а именно площадь, мы оценили и подтвердили гигиеническое состояние полости рта у лиц которые использовали ортопедические конструкции. Применение метода ирригации тканей пародонта для несъемных ортопедических конструкций приводит к уменьшению процентной части площади белковых фракталов цифровых образцов кристаллографии. Результаты подтверждены статистическими и клиническими данными.

Ключевые слова: микроскопическая кристаллография, ирригация, полость рта, пародонт, металлокерамические несъемные ортопедические конструкции.

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RETROSPECTIVE ANALYSIS OF PRIMARY FORENSIC MEDICAL EXAMINATIONS OF THE LOWER EXTREMITIES MECHANICAL TRAUMA

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The purpose of the study was to perform a retrospective analysis of primary forensic medical examinations that established moderate severity of bodily injuries in victims with mechanical injuries of lower extremities. The study revealed the predominance of road traffic injuries (92%); 8% were falls from the own height. Road traffic injuries were characterized by a predominance of people at working age (73%); vehicle hitting a pedestrian (86%); getting injured with polytrauma (82%); formation of diaphyseal fractures of the thigh and shin (78%); the presence of comorbid diseases (61%). The authors consider it necessary to revise the existing and to substantiate new medical criteria for determining the severity of bodily injury, taking into account the features of connective and muscle tissues reparative regeneration.

Key words: mechanical trauma of the lower extremities, forensic medical examination, fractures of long bones, road traffic injury.

The work is a fragment of the research project "Forensic substantiation of morpho-clinical criteria for expert assessment of bodily injuries, determination of limitation period and cause of death", state registration No. 0118U000951.

Forensic medical examination of living individuals is the most common type of expert activity. Establishment of damages and evaluation of their severity, in order of frequency, occupies the 1st place in forensic practice and is carried out both in criminal and civil proceedings [4]. According to data obtained from Kharkiv Regional Bureau of Forensic Medical Examination (KRBFME), over the last 5 years, the number of examinations of living individuals has increased from 83.7% in 2015 to 91.2% in 2019 (compared to all examinations and examinations of victims). Numerous works were dedicated to the examination of living persons, in particular, to the injuries' severity determination [1, 3, 15].

Mechanical trauma of the lower extremities leads to damage to both soft tissues (tendons, ligaments, fascia, articular bag, sarcolemma and perimisia of muscle fibers, etc.) and supportive (bone, cartilage) types of connective tissue [11]. Each of them has a different resistance to the traumatic factor. Accordingly, the standard terms of reparative regeneration, after mechanical damage to each type of connective tissue, varies drastically – from 3-5 weeks in cases of tendon ruptures, up to 4-5 months (without callus remodeling) in cases of long tubular bones' fractures.

It should be noted that traumatic diaphyseal fractures of long tubular bones are accompanied by ruptures of intraosseous vessels, damage to fascia, ruptures and imbibition of muscle fibers. That can lead to the development of persistent contractures of adjacent (to a fracture zone) joints, regardless of the treatment method (surgical or conservative) [11]. In addition, metaepiphyseal fractures are also accompanied by ruptures of periarticular tissues (joint bag, ligaments, muscle tendons), damage to the hyaline articular cartilage, development of hemarthrosis with the formation of post-traumatic contractures of the injured joint [10]. In this regard, the terms of callus formation [6], and, especially, the periods of the rehabilitation in patients with fractures of long tubular bones [7], can significantly exceed the recovery time after isolated (especially partial) ruptures of ligaments or tendons.

Determining the severity of bodily harm, in particular, after a mechanical injury to the lower extremities, which is not life-threatening, is carried out in accordance with paragraphs 2.2.1 of "Rules for the forensic medical determination of bodily harm severity" in the Order No. 6 of the Ministry of Health of Ukraine dated 01/17/95. The main criterion, in these cases, is the duration of a health disorder for a period of 21 days, the excess of which permits the expert to evaluate them as the moderate bodily harm. Based on this, injuries that are not comparable by their nature, the duration of the treatment, and, in some cases, their outcome, are evaluated by the same criteria: insulated rupture of the periarticular ligament (for example, rupture of medial collateral ligament of the knee joint) and the intraarticular fracture (for example, tibial epimetaphysis) with displacement. We presume that evaluation of the bodily injuries severity during a forensic medical examination of non-life-threatening mechanical injuries of the lower extremities should be justified basing on the objectively established fact of a violation in the structure and function of the body (in particular, musculoskeletal system) mainly in cases of damage outcomes, rather than basing on the formal duration of hospital stay and the term of outpatient treatment.

The purpose of the study was to perform a retrospective analysis of primary forensic medical examinations that established moderate severity of bodily injuries in the short post-traumatic period in victims with mechanical injuries of lower extremities. This analysis is aimed to clarify the nature of the lower extremities damage.

Material and methods. *Study design* is a retrospective cohort study.

Material of the study was the total of 100 reports on primary forensic medical examinations of victims with moderate severity of bodily injuries resulting from mechanical trauma to the lower extremities. In all cases, an expert assessment was carried out in the Kharkiv Regional Bureau of Forensic Medical Examination (KRBFME), within a period of not more than 1 month after an injury. Reports of primary forensic medical examinations were selected by random sampling, for the period February – June 2018.

Inclusion criteria – isolated ruptures of soft tissues (muscles, tendons, ligaments, menisci of the knee joint) of the lower extremities, isolated fractures, dislocations and fractures with dislocations of the femur or shin bones, multiple and combined non-life-threatening injuries, in which mechanical trauma of lower extremities prevailed, which led to moderate severity of bodily injury and which were assessed no later than in 1 month from the day of injury.

Exclusion criteria: isolated mechanical trauma of lower extremities, as well as multiple or combined injuries, which were regarded as light or severe injuries, or were performed in terms exceeding 1 month after injury.

Research methods were the retrospective analysis, statistical method.

When carrying out a retrospective analysis, the following indices were studied: trauma mechanism; type of injury (open, closed); the nature of the injury (isolated, multiple, combined); the nature of damage to the lower extremities tissues (tears of soft tissues: menisci, ligaments of the knee and ankle joints; fractures of the femur and lower leg bones); nature of the fracture (medial / lateral fractures of the femoral neck, transtrochanteric fractures, diaphyseal fractures, epimetaphysis fractures of the distal femur / proximal tibia, fractures of the medial / lateral ankle); the presence of dislocation / subluxation of the hip / knee / ankle joints. In the case of road traffic injuries, mechanical injuries of the lower extremities were identified in active (driver) and passive (passenger, pedestrian) road users.

When assessing the somatic status of the victims, the presence of comorbid conditions was taken into account: coronary artery disease, hypertonic disease, angina pectoris, obesity, diabetes mellitus, cholelithiasis, chronic gastroduodenitis.

Data processing was performed using descriptive statistics. The average age of the victims was determined by the formula $M \pm m$, where M is the arithmetic mean, m is the standard error of the mean.

Results of the study and their discussion. The results of the analysis on the parameters of lower extremities' mechanical trauma, which, according to the results of the primary forensic medical examinations was accompanied by moderate bodily injuries, made it possible to establish the following.

The mean age of the victims was 44.0 ± 17.8 years (18-81 years). The largest number of victims was at the working age of 18-60 years old – 73% (73/100); of injured older persons (61-81 years old) there were 27% (27/100). There was a slight prevalence of males (53% of 100 patients), which in relation of the men to women ratio was 1.1: 1 (table 1).

More than half of the victims (61% of 100 patients) had comorbid diseases. The most common were hypertension (23% of 100 patients), obesity (15% of 100 patients) and coronary heart disease (11% of 100 patients). Diseases of the gastrointestinal tract were observed much less frequently (chronic gastroduodenitis and cholelithiasis at 3% (3/100)). Only 1 victim out of 100 cases (1%) had angina pectoris (table 1).

Injuries were generally caused by traffic accidents – 92% (92/100). Among other mechanisms of injury, there were: fallings from the own height with a rotated tibia (7%; 7/100) or foot (1%; 1/100). Isolated ruptures of the periarticular ligaments of the knee and ankle joints, which resulted in moderate bodily

injuries, were observed much less frequently among mechanical injuries of lower extremities – 6% (6/100) and 2% (2/100), respectively, and formed as a result of a fall from a height of their own growth.

Table 1

Distribution by age, gender, frequency of comorbid diseases in patients with moderate degree of bodily injuries due to mechanical injuries of lower extremities

Parameters	Number of victims (n = 100)		Parameters	Number of victims (n = 100)	
	abs.	%		abs.	%
Age			Comorbid diseases:		
18 - 60 years old	73	73	- hypertonic disease	23	23
61 - 75 years old	16	16	- obesity	15	15
over 76 years old	11	11	- coronary artery disease	11	11
Mean age 44,0 ± 17,8 years old			- diabetes mellitus	5	5
Gender			- chronic gastroduodenitis	3	3
- male	53	53	- cholelithiasis	3	3
- female	47	47	- angina pectoris	1	1

To clarify the mechanism of road traffic injury, all 92 victims of road accidents were taken as 100%. It was found that the most often, by collision of person with a moving car – 86% (79/92). Damage to a passenger (7%; 6/92) and a driver (3%; 3/92) in a passenger compartment, a collision of a person with a moving motorbike (2%; 2/92), and damage from a driver falling out of a moving motorcycle (1%; 1/92) were much less common (table 2). Thus, active road users (drivers) participated in 4% (4/92) of road accidents, passive participants (pedestrians and passengers) – in 96% (88/92).

Table 2

Nature of injuries and road traffic injury mechanism in patients with moderate bodily injuries

Parameters	Number of victims (n = 92)	
	abs.	%
Polytrauma	75	82
- combined injury	66	72
- multiple fractures	9	10
Isolated mechanical injury	17	18
Open damage	27	29
Closed damage	65	71
Injury mechanism		
- collision of a man with a moving car	79	86
- damage to a passenger in a car cabin	6	7
- damage to the driver in the car cab	3	3
- damage from a driver falling out of a moving motorcycle	2	2

According to our material, the mean age of the road accidents victims was 41.5±18.8 years; persons of working age prevailed (73% of 92 patients). The ratio of male to female was 1.1:1.

As it is noted above, according to our material all the long bones fractures of the lower extremities were obtained as a result of road traffic accidents (RTAs), which were 92% (92/100) of the lower extremities mechanical injuries. In this group of 92 patients as a result of a road traffic injury, predominantly combined injuries were observed (72%; 66/92) (table 2), in which bone fractures were the leading lesion; closed fractures predominated (71%; 65/92). Fracture of the femur and / or lower leg bones in all cases was accompanied by bruised and lacerated wounds, bruises, abrasions, and subcutaneous hematomas of the head, and/or trunk, and/or upper limbs. Closed head injury with a mild degree concussion of the brain was diagnosed in 23% (23/92) of cases. Also, in 3% (3/92) of cases, closed fractures of the ribs were revealed; in one victim - self-healing dislocation of the humerus, in 3% (3/92) – diaphyseal fractures of the forearm, in 7% (7/92) – distal radial bone fractures, in 2% (2/92) – fractures of the ulna styloid process.

With multiple fractures of lower extremities (10%; 9/92), ipsilateral and contralateral fractures of the femur and tibia occurred with almost the same frequency – 4% (4/92) and 3% (3/92) of cases, respectively. In 2% of cases (2/92), a double fracture of the leg bones was revealed (table 3).

According to the nature of the damage, diaphyseal fractures of long bones predominated, which together accounted for 78% (72/92) of the cases. Of these, femoral diaphysis fractures accounted for 32% (29/92), diaphyseal fractures of the tibia – 47% (42/92). Intra-articular fractures of various localization were formed in 26 injured in traffic accidents. In this case, the hip joint suffered less frequently than others: medial fractures of the femoral neck were recorded in 4% (4/92) cases. Intra-articular fractures of the knee and ankle joints formed with the same frequency – 11 observations: with damage to the distal femur epimetaphysis – 2% (2/92), proximal tibia epimetaphysis – 10% (9/92), internal / external ankle fracture – 3% (3/92), both ankles – 9% (8/92) (table 3).

According to the data of the primary forensic medical examinations that were studied, a road traffic pedestrian injury in a collision with a moving car (78%; 62/79) was noted in the houses courtyards. Another 2

cases (out of 92) of a pedestrian injury in a collision with a moving scooter were registered in a city road near a traffic light. Other accidents involving collisions of moving cars (9 out of 92) occurred at unregulated intersections of secondary urban roads. A road traffic injury with a passenger who fell out of a motorcycle that started moving (2 out of 92) was observed in rural roads. In all these cases, road accidents were characterized by a low vehicle speed (30-40 km/h) and, accordingly, a relatively small amount of kinetic energy absorbed by the body of the injured road user. Due to this circumstance, the injuries received by the victims of the road accident were harmless to life and were characterized by a moderate severity of bodily injuries.

Table 3

Nature of long bones damage to the lower extremities due to mechanical injuries with bodily injuries of moderate severity

Nature of damage	Number of victims (n = 92)	
	abs.	%
Medial fractures of femoral neck	4	4
Transtrochanteric fracture	1	1
Femoral diaphysis fracture	29	32
Fracture of the distal femur epimetaphysis	2	2
Fracture of the proximal tibia epimetaphysis	9	10
Diaphyseal fracture of the shin bone	43	47
Double diaphyseal fracture of the tibia	2	2
Fracture of the medial / lateral ankle	3	3
Fracture of both ankles	8	9

In pedestrians, damage to the integumentary system of the lower extremities occurred during the first phase of a collision of a car, at the moment the pedestrian's body touched the surface of the car in a collision, during which the victim received a strike. In the same phase, bumper fractures of the diaphysis of the femur or shin bones were formed, which had a transverse fracture plane and, as a rule, were finely splintered. Contusions, abrasions and subcutaneous hematomas of the head and extremities, as well as fractures and dislocations of the bones of the upper extremities occurred mainly when the victim fell onto the road surface or the side of the road from the height of the car hood (the third phase of collision). Damage to the integumentary system in the second phase of the collision (pedestrian falling onto the hood of a car) due to a relatively low kinetic energy of the collision was noted only in 4 (out of 79) victims.

For drivers and passengers of cars, bruises and abrasions of the head area (impact on the side glass), bruises of the chest area and fractures of the ribs (impact on the steering wheel), bruises, hematomas and abrasions of the knee and ankle area, intra-articular fractures of the knee and fractures of the ankles (impact on the dashboard). All injuries were sustained in the phase of collision of the body with parts and components of the car's cabin. It should be noted that in both pedestrian and intra-salon trauma, the victims did not reveal the classic specific stamp-damage of the integumentary system from impact on the protruding parts of the car. This fact is largely due to the modern design features of passenger cars - the absence of protruding parts.

The absence of specific stamp-damages in a modern car injury makes it difficult to reliably reproduce the mechanism and circumstances of an accident during a forensic medical examination. Thus, in one of the expert reports that were analyzed, the mechanism of the formation of a medial fracture of the femoral neck was not finally determined when differentiating between a high-energy fracture due to a pedestrian injury and a low-energy fracture as a result of falling from a height of victim's own growth.

In the studied acts of primary forensic medical examinations (within 1 month after injury), the outcomes of fractures of the lower extremities are not presented, since at such times the union of fractures of the femur or tibia cannot be achieved, especially in patients with polytrauma. With respect to medical criteria, assessment of the treatment outcome is the normal course of the post-traumatic period. However, during a forensic examination, the body's response in the acute period of injury or at rehabilitation stages can prolong the duration of a health disorder - one of main criteria for assessing the bodily harm severity. We believe that there is an urgent need to review the current medical criteria for the forensic medical assessment of bodily injuries in mechanical injuries of musculoskeletal system and lower limbs in particular, which take into account the length of stay in inpatient or outpatient care. It is necessary to determine objective medical criteria that take into account current knowledge about the mechanism and nature of injuries, the features of the consolidation of various fracture types, the impact of concomitant injuries and comorbid diseases on reparative regeneration processes of connective and muscle tissues, the duration and content of the rehabilitation period.

One of this study results was the fact road traffic accidents significant prevalence in the structure of criminal injury. These data are consistent with the WHO statistics on road traffic injuries, which are one

of the most important medical, social and economic problems in the world. According to the WHO, every 5 min one person becomes disabled in the roads of the world [7]. Road traffic injuries are 35.4% of all types of injuries, they occupy the first place among the causes of death from mechanical damage, they are one of the main reasons for disabled citizens of working age and cost most countries 3% of their gross domestic product [15].

The data cited in the literature regarding the frequency of injury to men and women as a result of an accident are heterogeneous. Results close to the data of our study were obtained in the study of non-lethal car injuries with damage to long bones caused by collision with a car: 1.3:1 [1], 1.6:1 [2]. In contrast, in studies on the structure of accidents taking into account fatal outcomes, a significant predominance of males is revealed: 6:1 [5], 8.3:1 [9].

It should also be noted the presence of comorbid diseases, which were detected in more than half of the victims (61%; 61/100) (table 2). Although chronic diseases were not taken into account when conducting a forensic medical examination of people who had suffered from mechanical injuries of lower extremities, their presence, especially diabetes mellitus, obesity, arterial hypertension, can significantly affect the rate of formation and quality of callus and is a risk factor for delayed consolidation and non-fusion fracture [14].

Pedestrian collision in our study was the most frequent type of road accident (86%; 79/92), which coincides with the literature data [8]. Despite the fact that in most cases the pedestrian collision with a passenger car was characterized by a low speed of movement of the car, the main type of damage was polytrauma. In such cases, polytrauma is accompanied by a systemic inflammatory response syndrome (SIRS) [13], what has a negative effect on the processes of reparative regeneration of bone tissue [6]. It should also be noted that 85% of patients with combined trauma with injuries of the musculoskeletal system [12] and, as a rule, all patients with fractures of long bones of the lower extremities, regardless of the method of treatment (surgical, conservative), need rehabilitation.

Prospects for further research - in order to justify medical criteria for determining the severity of bodily damages during mechanical injury of long bones, a retrospective analysis of the commission of forensic medical examinations of injuries seems promising.

Conclusions

1. In the structure of mechanical injuries of the lower extremities in victims with moderate degree bodily injuries, the leading injuries were fractures of the femur and lower leg bones (92%) resulting from road traffic injuries.

2. In victims of road traffic injury in 61% of cases concomitant comorbid diseases were detected. In the structure of fractures, diaphyseal fractures prevailed - 79%, intra-articular fractures of the hip, knee and ankle joints formed in 27%; in 82% polytrauma was detected. The duration of a health disorder with these injuries will significantly exceed the period of 21 days, a priori established as the boundary between bodily injuries of mild to moderate severity.

3. We consider it is necessary to justify new medical criteria for assessing the severity of bodily injuries that will make it possible to objectively evaluate post-traumatic structural changes in the body and their functional consequences.

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Реферати

РЕТРОСПЕКТИВНИЙ АНАЛІЗ ПЕРВИННИХ СУДОВО-МЕДИЧНИХ ЕКСПЕРТИЗ ПРИ МЕХАНІЧНІЙ ТРАВМІ НИЖНІХ КІНЦІВОК

Сокол В.К., Колесніченко В.А., Проценко О.С.

Мета роботи - ретроспективний аналіз первинних судово-медичних експертиз, в яких встановлено середню ступінь тяжкості тілесних ушкоджень у потерпілих з механічними травмами нижніх кінцівок. Виявлено переважання дорожньо-транспортної травми (92%); в 8% відбулося падіння з висоти власного зросту. Дорожньо-транспортна травма характеризувалася переважанням осіб працездатного віку (73%); найздом транспортного засобу на пішохода (86%); отриманням політравми (82%); утворенням діафізарних переломів стегна і голілки (78%); наявністю коморбідних захворювань (61%). Автори вважають за необхідне перегляд існуючих і обґрунтування нових медичних критеріїв визначення ступеня тяжкості тілесних ушкоджень з урахуванням особливостей репаративної регенерації сполучної та м'язової тканини.

Ключові слова: механічна травма нижніх кінцівок, судово-медична експертиза, переломи довгих трубчастих кісток, дорожньо-транспортна травма.

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РЕТРОСПЕКТИВНИЙ АНАЛІЗ ПЕРВИЧНИХ СУДЕБНО-МЕДИЦИНСКИХ ЕКСПЕРТИЗ ПРИ МЕХАНІЧЕСКОЙ ТРАВМЕ НИЖНИХ КОНЕЧНОСТЕЙ

Сокол В.К., Колесніченко В.А., Проценко О.С.

Цель работы - ретроспективный анализ первичных судебно-медицинских экспертиз, установивших среднюю степень тяжести телесных повреждений у потерпевших с механическими травмами нижних конечностей. Выявлено преобладание дорожно-транспортной травмы (92%); в 8% произошло падение с высоты собственного роста. Дорожно-транспортная травма характеризовалась преобладанием лиц трудоспособного возраста (73%); наездом транспортного средства на пешехода (86%); получением политравмы (82%); образованием диафизарных переломов бедра и голени (78%); наличием коморбидных заболеваний (61%). Авторы считают необходимым пересмотр существующих и обоснование новых медицинских критериев определения степени тяжести телесных повреждений с учетом особенностей репаративной регенерации соединительной и мышечной ткани.

Ключевые слова: механическая травма нижних конечностей, судебно-медицинская экспертиза, переломы длинных трубчатых костей, дорожно-транспортная травма.

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THE MOST SIGNIFICANT MORPHOLOGICAL FEATURES OF THIRD MOLARS IN ADULT PERSONS ACCORDING TO ORTHOPANTOMOGRAPHY

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The article presents a theoretical generalization and a new solution of the scientific problem, which consists in determining the morphological features of third molars according to orthopantomography. A literature review, the analysis results of the dentition orthopantomograms of persons in the first period of mature age, randomly selected in the clinic, are presented. A systematic analysis of the general visual representation of third molars' morphological features in people of mature age, was carried out based on orthopantomograms selection. Morphological features of third molars, forms of retention, types of dystopia, morphological differences between impacted and embedded third molars were established. The systematic approach used in the work permitted to put in order the variational polymorphism of anomalously developed third molars' forms, while identifying the most indicative signs for their classification.

Key words: teeth, third molars, orthopantomogram, retention, dystopia.

The work is a fragment of the research project "Age aspects of the structural organization of the immune system, the gastrointestinal tract and genitourinary system glands in norma and in pathology", state registration No. 0116U004192.

Normally, as it is known, the eruption of the third molars completes the process of odontogenesis, as a result of which these teeth occupy the most extreme place in the remaining alveolar processes of the jaws [3, 4, 7]. They should be considered late not only because they erupt in delayed time, but also due to the late dates of their formation. This usually occurs in the 5th year of postnatal life, ie about one year before the complete eruption of the first permanent molar [3, 5, 8]. Naturally, the rudiments of the third molars go through the same stages of their development as other teeth, resulting in the formation of their crown sections, and then it is the turn of root development, which is associated with the process of teething. It should be noted that from the age of 8 calcification of the masticatory surface of the crown begins, at the

age of 12 the intramaxillary formation of the crown ends, and the period of formation and growth of roots and periodontium begins after the eruption of permanent teeth [5, 6, 8].

Unlike deciduous teeth, the eruption of which is associated with the process of resorption of the roots of the respective teeth, large molars have an obstacle in the way of their eruption in the form of bone tissue [1, 2, 5]. Regarding this process, there is an idea that the destruction of this bone barrier is due to mechanical pressure on the crowns, which grow due to the elongation of the roots. This point of view cannot be considered correct, because it is purely mechanical and does not take into account the fact that in a living organism all the driving forces are generated by the activity of certain cellular structures. The forming cells in this process are odontoblasts and cementoblasts, which form the roots of the teeth, while the destruction of bone tissue of the alveolar processes is carried out by osteoclasts. Normally, their activity should be somewhat ahead of the process of root formation in order to provide space for the promotion of their crowns. Perhaps as a result of violation of this process, the third molars are most often prone to various individual deviations from the genotype-given shape, characteristic of large molars [1, 2, 3]. In this regard, in dentistry, common terms such as "retention" and "dystopia", which are often used in an equivalent sense [3, 5, 8]. But, if we analyze them in the original content, then such interchange is illegal, because the first term means "hold" or "stop", and the second – "permutation" or "shift". In this formulation of the question, that is, in a legible attitude to the terms that mean different forms of abnormal development of third molars, there are no attempts in the literature to thoroughly analyze them. In addition, in the category of "dystopia" in dentistry, the term "impact" is used, which refers to a formed tooth that has not erupted and rests its crown on another tooth [2, 6, 7]. These data are still insufficiently explained in the literature.

It is reported that various forms of abnormal development of third molars most often occur in the lower jaw [4, 6, 7], mainly in women [8].

Given the large number of anomalies in the formation and eruption of third molars, this issue remains relevant for both modern therapeutic dentistry and maxillofacial surgery [3, 4, 6].

The optimal method of studying the morphological features of teeth, especially in eruption anomalies is orthopantomography [6, 7, 8].

Given the above, we set a goal to conduct a systematic analysis of abnormal development of third molars, which are most common in people of the first period of adulthood on the basis of analysis of orthopantomograms.

The purpose of the study was to perform the system analysis of the third molars' morphological features in persons of mature age according to orthopantomography.

Materials and methods. To solve this purpose, 40 orthopantomograms of men and women of the first period of adulthood were used, which were selected at random. The data were obtained in the Department of Surgical Dentistry of the Regional Dental Clinic and at the Department of Surgical Dentistry and Maxillofacial Surgery with Plastic and Reconstructive Surgery of the Head and Neck of the Ukrainian Medical Stomatological Academy (Poltava).

Orthopanthograms of patients were randomized into 2 groups, 20 in each:

Orthopanthograms of men aged 22 to 35 years

Orthopanthograms of women aged 21 to 35 years

A simple visual approach to analysis was used for them.

Using radiological methods, we obtained data on the spatial state of the dental system, location and depth of retained and dystopian teeth in the thickness of the alveolar process [7]. We evaluated the condition of the coronal, root part of the teeth and bone tissue of the jaws, position, size and shape of the upper and lower third molars, the number of roots and the degree of their formation, and assessed the total longitudinal dimensions of the upper and lower third molars.

We analyzed the configuration of the third molars on the basis of a visual assessment of the shape of their coronal and root divisions.

The position of the third molars was determined by the orientation of the crown in the jaws.

The total longitudinal dimensions of the upper and lower third molars were determined by estimating the length of their root and the height of the crowns.

The obtained data of sample groups are clearly presented on orthopanthograms.

This method of evaluation of orthopantomograms does not involve obtaining metric parameters.

Results of the study and their discussion. First of all, we noticed that the incised third molars in males and females of the first period of adulthood are a disordered alternation of extremely different in size and shape of the teeth, which is expressed in different combinations of configurational relationships between the crown and root parts and depends from the opposite in the bite and sex, but does not correlate

with age. Obviously, this is an external manifestation of individual (phenotypic) features of their development.

Numerous individual features of third molars are expressed in their size, general shape, number of roots and configuration of coronal divisions. In this regard, there is a description in the literature of many such different morphological features but we have identified many variable forms that arise as a result of a combination of these individual features.

It is quite natural that we were interested in the third molars presented in the orthogonal projection, which erupted in their size in relation to other large molars, which allows us to clarify the reliability of some of the above data. It is clear that in such a projection, not all morphological features of the teeth were available for objective assessment. Nevertheless. Based on these images, we could see that the crowns of all molars have approximately the same height, which was significantly less than its width in the mesio-distal direction. It is also significant that the width of the crowns of the lower molars is slightly larger than the width of the upper ones (Fig. 1). But to a greater extent, large molars differ in shape and, especially, in the length of their root sections. Evaluation of orthopantomograms clearly confirms our rule: "if the height



Fig. 1 Permanent dental occlusion of a patient aged 22 years (orthopantomogram).

of the crown of these teeth is a constant value, the length of their root is a variable value", and therefore the total longitudinal size of the tooth depends entirely on the latter indicator. In fact, this is expressed in the fact that the lower molars in longitudinal dimensions look more developed than the upper ones (fig. 1).

In this case, sexual dimorphism is manifested in a slightly smaller size of female wisdom teeth. In addition, regardless of the opposite in the tooth bite, quite often there is a relationship between the longitudinal size and the time of eruption. Thus, in each row in the mesio - distal direction, they are arranged as if by rank, so that the first molar is the longest and the third the shortest.

It should be noted that random samples of orthopantomograms of the dental system of men and women of the studied groups indicate a large phenotypic diversity of dental occlusion, which in some cases is normal, in others - is considered abnormal. Apparently, this is due to the fact that as a result of developmental disorders of the third molars are most often prone to various individual deviations from the genotype-given form. The most common "retention" and "dystopia" of third molars. Thus, among all the orthogonal images of the dental system studied by us, there are many individual options that allow us to make sure that adults often have cases of abnormal location of wisdom teeth. At the same time, some of them illustrate the intra - maxillary placement of the formed tooth, others – violation of its formation at the earliest stages of development, to the point that in some cases there are not even traces of their rudiments (fig.2).



Fig. 2 Permanent dental occlusion in a patient aged 24 years with developmental disorders 18, 28, 38, 48. On the upper jaw there are wisdom teeth, which are in a retained state, on the lower – the absence of their formation (orthopantomogram).

In other cases, the third molars are in the retained state at the stage of histogenesis of crowns symmetrically lower and upper dentition (fig. 3).

However, there are often cases of different variants of their asymmetric retention (fig. 4 A).

At the same time, we pay attention to the fact that in the vast majority of third molars with a delay in their development are contained in the jaws in a kind of follicular cells, the wall of which is formed by a thin layer of compact bone, and between it and the most underdeveloped tooth is a relatively wide periodontal gap. (fig. 2, 3). In our opinion, this morphological feature can be attributed to the distinctive feature of retained teeth. Unfortunately, at present we cannot find out what these periodontal structures are,

as this requires a special morphological study of the whole preparations of the corresponding jaws, which are extremely difficult to obtain. However, it can be assumed that these periodontal structures are the result of the transformation of the dental sac, which is formed from the mesenchyme surrounding the enamel organ at the earliest stage of odontogenesis.

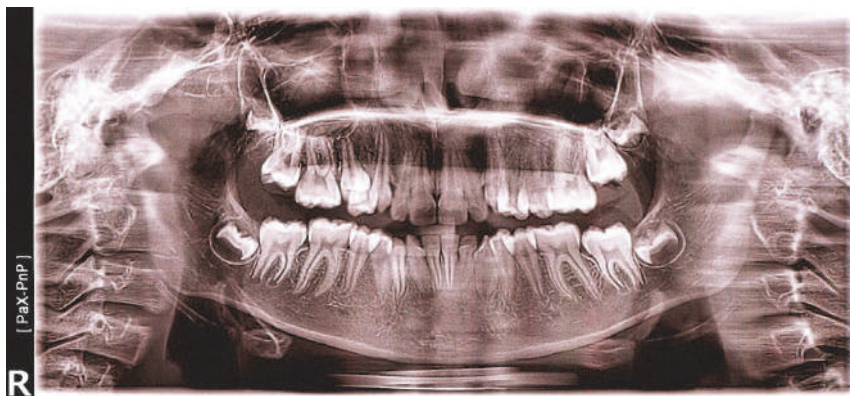


Fig. 3 Permanent dental occlusion of a patient aged 22 years with developmental disorders 18, 28, 38, 48. There are follicular cells in which there are retained teeth (orthopantomogram).

the crown of the tooth is due to the need to isolate the enamel from the surrounding connective tissue of the bone base of the jaws, in which there are immunocompetent cells capable of responding to enamel autoantigens.

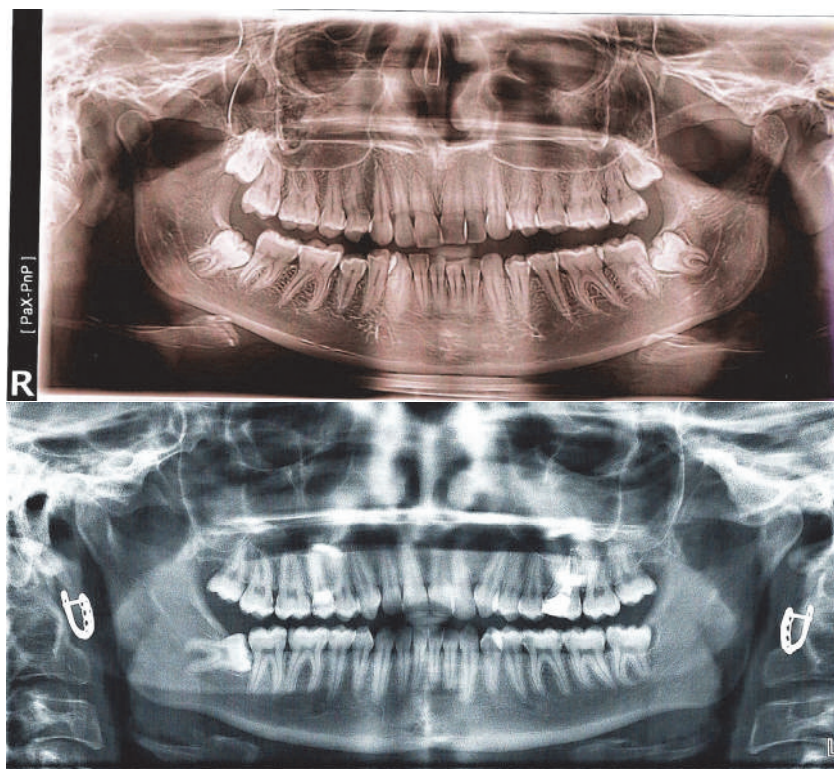


Fig. 4. A. Permanent dental occlusion in patients aged 21 years with developmental disorders 18, 28, 38, 48. On the upper and lower jaws there are wisdom teeth that are in a retained state (orthopantomogram). B. Dystopia 48: medial position of the affected 48 in a patient aged 35 years (orthopantomogram).

System analysis of orthopantomograms showed that "retention" and "dystopia" of third molars are most common in the clinic and do not have a predominant localization in the lower jaw, which contradicts the data of some researchers [5, 7, 8] and does not depend on gender as previously thought [8]. It is obvious that in such a statement of the question these facts are in contradiction with each other.

Thus, we have limited ourselves to a brief review of a certain sample of orthopantomograms of the dental system of adults. This is due to the fact that we did not seek a detailed description of the abnormal development of the third molars, the whole variety of variants of shapes and positions of the third molars. Dealing with these questions, we realized that random samples of different individual X-ray images of the dental system, we can not trace in a series of observations the relevant stages of their development and, moreover, note the various deviations from their normal formation. Unlike other similar studies, we aimed to systematize this diversity to clarify some controversial provisions in dentistry.

In contrast to the retained third molars for their dystopian analogues, ie those that are in the jaws in the formed state, but in the displaced position, according to our observations, it is not typical to have such a continuous periodontal structure. Around their roots is a normal periodontal fissure, which expands only around the crown. It can be assumed that its belonging to

Given that the practical need to obtain orthopantomograms, which we used in our studies, were certain clinical indications, it is clear why many of them often have third molars in a dystopian state and in a very different position. Among them, there are only inverted ("upside down") options. According to the orientation of the crown in the jaws, we have identified the following positions: 1 – buccal; 2 – lingual – palatine; 3 – medial and 4 – distal. The last two variants of dystopia of the third molars should be attributed to their impact position. Thus, in the medial direction they on a tangent or at right angles rest a crown in the second molar, and in distal – the second molar serves as a support for their roots (fig. 4 B).

Conclusions

In this regard, we consider it appropriate to take into account the following conclusions:

1. cut third molars are extremely diverse in size and shape, which is expressed in a different combination of configurational relationships between the coronal and root parts, which depends on the opposite in the bite and sex, but does not correlate with age;

2. all forms of abnormal development of third molars that occur in the clinic should be divided into two categories that differ in the nature of morphogenetic disorders. The first of them should include all forms of retention, which should be understood as a delay or cessation of tooth development at the stage of formation of its crown, and the second category – various deviations of the longitudinal orientation of the tooth at the bone base of the jaws. called dystopia;

3. we first drew attention to the fact that retained teeth are contained in the jaws in a kind of follicular cells, the wall of which is represented by a plate of compact bone, between which the most underdeveloped tooth is a relatively wide gap. In contrast, dystopian teeth have similar formations only around the crown, while around the roots is a normal periodontal gap, ie periodontium;

4. among the dystopian third molars, according to the orientation of their crown in the jaws, the following positions are distinguished: 1 – buccal; 2 – lingual-palatine; 3 – medial and 4 – distal. The last two variants of dystopia of the third molars should be attributed to their impact position. In this case, in the medial direction, they rest on the crown in the second molar, and in the distal – the second molar serves as a support for their roots.

Prospects for further research lie in the fact that we plan to perform a comparative characterization of the third molars' polymorphism in the age aspect by gender and to establish the pattern of caries.

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Реферати

**НАИБЛЬШ ЗНАЧИМІ МОРФОЛОГІЧНІ
ОСОБЛИВОСТІ ТРЕТІХ МОЛЯРІВ
У ЛЮДЕЙ ЗРІЛОГО ВІКУ ЗА ДАНИМИ
ОРТОПАНТОМОГРАФІЇ**

Талаш Р.В.

У статті викладено теоретичне узагальнення і нове вирішення наукового завдання, яке полягає у визначенні морфологічних особливостей третіх молярів за даними ортопантомографії. У роботі представлено огляд літератури, результати аналізу ортопантограм зубощелепної системи у людей першого періоду зрілого віку, відібраних довільно. Системний аналіз загального візуального уявлення про морфологічні особливості третіх молярів у людей зрілого віку, здійснений нами на основі добірок ортопантограм. Встановлено морфологічні особливості третіх молярів, форми ретенції, види дистопії, а також морфологічні відмінності між ретенваними і дистопованими третіми молярами. Використаний у роботі системний підхід дозволив привести до визначеного порядку варіативну поліморфність форм аномально розвинутих третіх молярів, виділивши при цьому найбільш показові ознаки для їх класифікації.

Ключові слова: зуби, треті моляри, ортопантомограма, зубощелепна система, ретенція, дистопія.

Стаття надійшла 12.09.2019 р.

**НАИБОЛЕЕ ЗНАЧИМЫЕ МОРФОЛОГИЧЕСКИЕ
ОСОБЕННОСТИ ТРЕТЬИХ МОЛЯРОВ
У ЛЮДЕЙ ЗРЕЛОГО ВОЗРАСТА ПО ДАННЫМ
ОРТОПАНТОМОГРАФИИ**

Талаш Р.В.

В статье изложено теоретическое обобщение и новое решение научной задачи, которое заключается в определении морфологических особенностей третьих моляров по данным ортопантомографии. В работе представлены обзор литературы, результаты анализа ортопантограмм зубочелюстной системы у людей первого периода зрелого возраста, отобранных произвольно. Системный анализ общего визуального представления о морфологических особенностях третьих моляров у людей зрелого возраста, осуществлен нами на основе подборок ортопантограмм. Установлены морфологические особенности третьих моляров, формы ретенции, виды дистопии, а также морфологические различия между ретенрованными и дистопированными третьими молярами. Исползованный в работе системный подход позволил привести в определенный порядок вариативную полиморфность форм аномально развитых третьих моляров, выделив при этом наиболее показательные признаки для их классификации.

Ключевые слова: зуби, треті моляри, ортопантомограма, зубочелюстная система, ретенция, дистопія.

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ADAPTATION OF POLYMERASE CHAIN REACTION-RESTRICTION FRAGMENT LENGTH POLYMORPHISM METHOD FOR POLYMORPHISM (RS2583988) ANALYSIS IN ALPHA-SYNUCLEIN GENE

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The article suggests the polymerase chain reaction-restriction fragment length polymorphism method for a single nucleotide polymorphism (rs2583988) detection for further prediction of Parkinson's disease risks. Based on the meta-analysis results for the single nucleotide polymorphism rs2583988 (C>T) in alpha-synuclein gene; current polymorphic variant was chosen by analyzing bioinformatical and genetic studies. We have designed a pair of primers and conditions for restriction analysis to detect different alleles in rs2583988. As a result, there was developed a simple and affordable method for detecting single nucleotide polymorphism rs2583988 alleles. The method is expected to be suitable for clinic laboratories, due to affordable cost and equipment, and to be used in further association studies.

Key words: Parkinson's disease, alpha-synuclein gene, single nucleotide polymorphism, polymerase chain reaction-restriction fragment length polymorphism.

The work is a fragment of the research project "Clinical, molecular genetics and neurophysiologic features of the course in various forms of Parkinson's disease", state registration No. 0119U102848.

Most of non-infection diseases develop as a result of complex gene interaction and the environmental influence. Parkinson's disease is a prototypical chronic condition and its risk increases with age [1, 11] that results to progressive disability [3], impairs driving ability [2]. A person, who was not born with the disease, may have a high risk of its further development. This is known to be the genetic predisposition or susceptibility. The genetic predisposition to a particular disease is associated with the presence of mutations in one or more genes and / or combinations of their alleles. Development of methods in molecular genetics has made it possible to detect a predisposition to one or another disease long before its clinical manifestations. Currently, it is believed that the key reason of Parkinson's disease pathogenesis is the presynaptic alpha-synuclein protein aggregation [8, 12]. Based on the meta-analysis results for the α -Synuclein Gene (*SNCA*) rs2583988 (C>T) polymorphic variant, significant associations with the development of Parkinson's disease have been established [4, 5], and the T-allele of the polymorphic variant of the *SNCA* gene rs2583988 is significantly spread in patients with cognitive impairments [7, 14]. The rs2583988 is located in the promoter region of the *SNCA* gene [6, 9].

The purpose of the work was to apply known methods for the polymorphism analysis of *SNCA* gene (rs2583988), though application of PCR-RFLP method hasn't been suggested yet.

Materials and methods. Samples of buccal epithelium were collected from patients with PD, hospitalized in the Neurological Department, M.V. Sklifosovsky Poltava Regional Clinical Hospital. The agreements for using biological materials for further analysis were taken from each individual.

Genotyping was performed at the LLC "Gentris LTD" Poltava laboratory. DNA was extracted using ion exchange resin Chelex-100 [13]. Selected samples were placed in labeled polypropylene tubes with a lid with a capacity of 1.5 cm³. To the contents of the tubes was added 0.1 cm³ of 20% Chelex-100 suspension and incubated for 6 h at +56° C. After shaking the vortex tubes, they were placed in the thermostat and incubated for 8 mins at +96° C. Samples of the DNA solution were stored at -20° C.

The allele determination of *SNCA* gene (rs2583988) was performed using PCR-RFLP. DNA amplification was performed using recombinant Taq DNA polymerase (Thermo Fisher Scientific), according to the manufacturer's recommendations. We have designed the following primers, using the Blast primer tool [10]: forward – CCATGACCTCCTTGAGACCT and reverse – TGCCAAAGGACTAACAAATTACC (Metabion international AG, Germany). Amplification was performed on programmable thermostat "Tercik-2" (DNA Technologies, RF) under the conditions of annealing oligonucleotide primers at 60 °C. The components of the PCR mixture used for DNA amplification in polymerase chain reaction were stored in a freezer at -20 °C. Before use, they were thawed at room temperature. The required quantity of clean polypropylene microtubes with a lid with a capacity of 0.5 cm³ was marked with serial numbers.

The Rsa I restriction of PCR products was carried out in accordance to the manufacturing instructions (Thermo Fisher Scientific). The hydrolyzed PCR products were separated by 8% polyacrylamide gel (SDS) electrophoresis in 1 × TBE buffer. To the tubes with samples of hydrolyzed PCR

products was added 6 × sample buffer and mixed thoroughly. 0.005 cm³ of molecular weight marker was added to the first well, and 0.01 cm³ of hydrolyzed PCR products were added to the following wells. DNA pUC19 / MspI (HpaII) (Thermo Fisher Scientific, USA) was used as a molecular weight marker. Electrophoresis was performed for 3 h at a current of 50 mA in a vertical electrophoresis chamber 20 × 20 cm (Cleaver Sci.Ltd omniPAGE Maxi, UK). After electrophoresis, the gel was stained with ethidium bromide solution (10 mg / cm³) for 3–6 min, the gel was washed three times with distilled water and the electrophoresis results were documented using a gel imaging system (MicroDOC with UV transilluminator UVTS312, UK).

Results of the study and their discussion. First of all, it should be noted that the Parkinson's disease (PD) is one of most common neurodegenerative diseases. One of the key reasons of Parkinson disease pathogenesis is the presynaptic alpha-synuclein protein aggregation. Based on this, the polymorphism of the gene encoding the presynaptic alpha-nucleic protein – SNCA gene, could affect the development of PD.

The SNCA gene was discovered to provide coding information to create a small size protein, called alpha synuclein. Alpha-synuclein was found to be a member of the synuclein family, which also included beta- and gamma-synuclein. It was detected in high amounts in the brain, and lower amounts in the heart, other muscles. In the brain, alpha-synuclein was found primarily at the ends of neurons in specialized structures called presynaptic endings. SNCA peptides happened to be major components of amyloid plaques in the brains of patients with Alzheimer's disease. Alternatively, spliced transcripts encoding different isoforms had been identified for this gene.

SNPs could act as biological markers, helping scientists find genes associated with disease. When SNPs occurred within a gene or in a regulatory gene, they might play a more direct role in disease by affecting gene function. Most SNPs didn't seem to affect health, however, some of these genetic differences had proven to be very important for the study of human health. Researchers had found SNPs that could help predict a person's response to certain medications, susceptibility to environmental factors such as toxins, and the risk of developing certain diseases. SNPs could also be used to track the inheritance of disease genes in families.

Current SNP rs2583988 was detected in the promoter region of the *SNCA* gene significant associations with the development of Parkinson's disease have been established. The rs2583988-*T* allele had significant associations with the development of Parkinson's disease. The rs2583988-*T* allele is more common in patients with cognitive impairments.

There had been relieved methods for the polymorphism analysis of *SNCA* gene (rs2583988):

Polymerase chain reaction in real time (Real-time PCR). Real-time qPCR has widely been used in many diagnostic areas, including the analysis of foodborne pathogens. There are certain benefits of real-time PCR, such as ability to monitor the progress of PCR, ability to accurately measure the amount and amplification and detection occurs in one tube excluding post-PCR manipulation;

DNA sequencing. In chain-termination PCR, the user mixes a low ratio of chain-terminating dNTPs in with the normal dNTPs in the PCR reaction. dNTPs lack the 3'-OH group required for phosphodiester bond formation; therefore, when DNA polymerase incorporates dNTP at random, extension ceases. The result of chain-termination PCR was found to be millions to billions of oligonucleotide copies of the DNA sequence of interest, terminated at a random lengths (n) by 5'-dNTPs. In manual Sanger sequencing, four PCR reactions are set up, each with only a single type of dNTP mixed in;

Sequenom MassArray iPLEX platform. The method for SNP genotyping described in this unit is based on the commercially available Sequenom MassARRAY platform. The assay consists of an initial locus-specific PCR reaction, followed by single base extension using mass-modified dideoxynucleotide terminators of an oligonucleotide primer which anneals immediately upstream of the polymorphic site of interest. Using MALDI-TOF mass spectrometry, the distinct mass of the extended primer identifies the SNP allele.

All the mention methods turned out to have several limitations, such as high budget of equipment and reagents, due to which they are hard to be implemented in Ukrainian laboratories for the temporary research. Polymerase chain reaction with further restriction enzyme analysis was chosen for the current SNP studies due to affordable cost and the equipment. Using PCR, specific sequences in a DNA was copied or "amplified" from many thousands to a million times using sequence-specific oligonucleotides, heat-resistant DNA polymerase and thermal cycling. Traditional (end point) PCR, detection and quantification of amplified the sequence is executed at the end of the reaction after the last PCR cycle, and include post-PCR analysis, such like gel electrophoresis and image analysis.

PCR theoretically was discovered to amplify DNA exponentially by doubling the number of target molecules for each amplification cycle. When it was first developed, scientists believed that the number of cycles and the amount of the final PCR product could be used to calculate the initial amount of genetic material compared to a known standard. To address the need for reliable quantification, a quantitative real-time PCR and endpoint PCR technique has been developed used mainly to amplify specific DNA for sequencing, cloning and use in other methods of molecular biology.

Among known methods the polymorphism analysis of *SNCA* gene (rs2583988) was not performed by PCR-RFLP yet. The *SNCA* gene sequence data (GenBank accession number AY079082) for designing the primers for SNP (rs2583988) was taken. Current SNP was analyzed for restriction site of any suitable enzyme. For detecting rs2583988 allele variants *Rsa* I enzyme was used; also, it is appropriate to use *Rsa* I isoshizomers (*Afa* I, *Csp* 6I, *Cvi* QI).

Primer-BLAST tool was used to design specific primers [6]. The program was developed at NCBI to help users make primers that are specific to the input PCR template. It used the program to design PCR primers and then submits them to BLAST search against user-selected database. The blast results were then automatically analyzed to avoid primer pairs that can cause amplification of targets other than the input template. From different primer variants the one with small amplification product, (173 bp) was chosen.

DNA oligonucleotides appeared to be essential components of PCR reaction. The key event of each oligonucleotide-based assay was the specific binding between oligonucleotides and their target DNA. However, single-stranded DNA molecules also tended to bind to unintended targets or themselves. The probability of such unspecific binding could increase with the complexity of an assay.

The received primers revealed no unexpected fragments, so the primers were picked up the right way. We expected that the digesting of the amplified DNA product with restriction enzyme *Rsa* I would allow to evaluate the cut of 173 bp DNA into 110 bp and 63 bp fragments.

Since a restriction enzyme might lose activity due to improper storage or handling, it is important to check the expiration date, verify that the enzyme had been stored at -20°C. The enzyme for activity by setting up a control reaction with 1 µg of standard control DNA, where you know that the DNA quality was high and the expected banding pattern. Enzyme had to be kept in frost-free freezers that undergo temperature fluctuations. It is also recommended to keep the enzymes in a cold rack in the freezer, as this helps to stabilize the storage temperature

The chosen conditions for PCR-RFLP method of SNP (rs2583988) analysis allowed determining the different alleles of the gene *SNCA* (fig. 1).

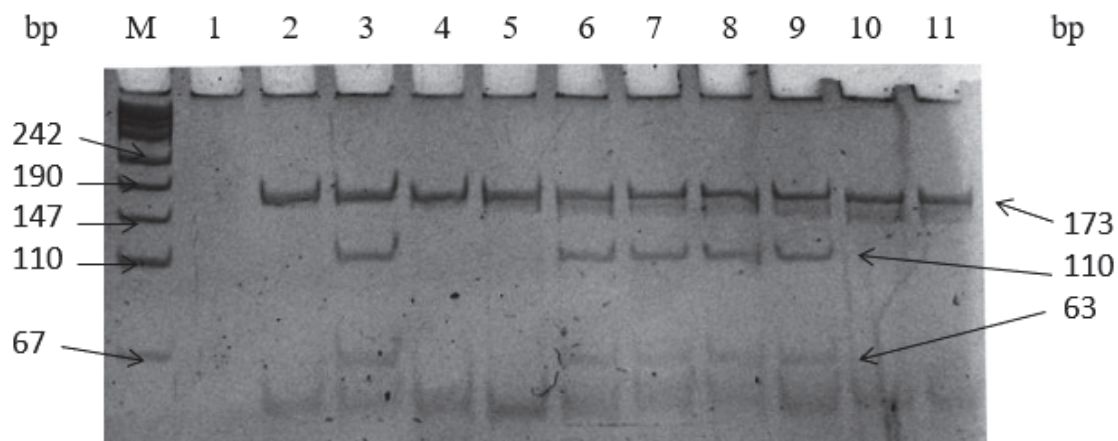


Fig. 1. M – *pUC19/Msp* I molecular weight marker, 1 – negative control (PCR without DNA), 2-11 – human DNA.

The resulting PCR amplification product sizes and enzymed DNA fragments completely coincided with the expected. It was shown that the detection of reaction products could be performed using electrophoresis in both 6% PAAG and 1.5% agarose gel (stained with ethidium bromide) using Tris-borate buffer (1 × TBE). The suggested conditions for PCR-PRLRF could be implemented to PCR laboratories.

It was essential to do SNP (rs2583988) the meta-analysis of previous studies [1, 2, 7, 14] to see the importance of its detection by comparing to the results in another populations. We found current SNP to be highly polymorphic in Brazilian, Canadian and American populations.

The first step for sample analysis should include DNA isolation. The isolation method including ion exchange resin Chelex-100, 20% was found to be the most convenient, comparing to DNA isolation methods including lysis solution and wash buffers [13]. For the convenient SNP analysis negative and positive controls were used during the experiment.

In order to achieve successful DNA amplification, we followed its importance to start off with the right primers. We took into account GC content to be 50%. The G and C bases have stronger hydrogen bonding and help with the stability of the primer. We avoided runs of 4 or more of one base, or dinucleotide repeats. Apparently, the designed primers in the Blast primer design program [10] met the expectations of the successful detection.

The developed method for detecting SNP (rs2583988) includes the primers and the PCR conditions. Not following the protocol could lead to wrong concentration of reagents and the reaction failure. We managed to complete the optimal reaction conditions, which were revealed in the methods.

Conclusion

After performing informational studies *SNCA* gene has been chosen as a candidate gene for further correlation studies with PD. Its SNPs are known to be influencing on PD development among the American population. From various SNPs of *SNCA* gene it has been selected SNP rs2583988 (C>T), which is located in the promoter region of the current gene. After the development of the primers and optimizing the conditions of PCR-RFLP method, the detection of SNP rs2583988 (C>T) turned out to be available. As a result of bio informational and experimental studies, we were able to implement a PCR-RFLP method for analyzing the *SNCA* gene polymorphism (rs2583988). Due to the affordable cost of reagents and equipment, the method can be applied in clinical and research studies in laboratories. Further associative studies with SNP genotypes and PD development should be conducted in the Ukrainian population to approve the correlation statistically. We assume that the method will allow predicting risks of PD and taking measures in a timely manner, preventing its development or facilitate its course. Also, it will allow taking into account the individual characteristics for providing the therapy. Further other SNPs in different regions of *SNCA* gene might be considered as relevant for the researches.

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Реферати

**РОЗРОБКА МЕТОДУ ПОЛІМЕРАЗНОЇ
ЛАНЦЮГОВОЇ РЕАКЦІЇ ДОВЖИН
РЕСТРИКЦІЙНИХ ФРАГМЕНТІВ ДЛЯ АНАЛІЗУ
ПОЛІМОРФІЗМУ (RS2583988) ГЕНА АЛЬФА-
СІНУКЛЕЇНА**

**Таряник К.А., Литвиненко Н.В., Олійниченко Є.К.,
Буслик Т.В., Почерняєв К.Ф.**

У статті запропоновано метод полімеразної ланцюгової реакції довжин рестрикційних фрагментів для виявлення однонуклеотидного поліморфізму (rs2583988) для подальшого прогнозування ризику хвороби Паркінсона. На підставі результатів мета аналізу

**РАЗРАБОТКА МЕТОДА ПОЛІМЕРАЗНОЇ
ЦЕПНОЇ РЕАКЦІЇ ДЛИН РЕСТРИКЦИОННЫХ
ФРАГМЕНТОВ ДЛЯ АНАЛІЗА
ПОЛІМОРФИЗМА (RS2583988) ГЕНА АЛЬФА-
СІНУКЛЕИНА**

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Буслик Т.В., Почерняев К.Ф.**

В статье предложен метод полимеразной цепной реакции длин рестрикционных фрагментов для выявления однонуклеотидных полиморфизмов (rs2583988) для дальнейшего прогнозирования риска болезни Паркинсона. На основании результатов цель анализа для

для однонуклеотидного поліморфізму rs2583988 (C> T) в гені альфа-синуклеїна. Поліморфний нуклеотид був обраний шляхом аналізу біоінформатичних та генетичних досліджень. Ми розробили пару праймерів та умови рестрикційного аналізу для виявлення різних алелів у rs2583988. Як результат, був розроблений простий і доступний метод виявлення однонуклеотидного поліморфізму алелів rs2583988. Очікується, що метод виявиться зручним для клінічних лабораторій, завдяки доступній вартості та обладнанню, а також використовуватиметься в подальших асоціативних дослідженнях.

Ключові слова: хвороба Паркінсона, ген альфа-синуклеїна, однонуклеотидний поліморфізм, полімеразна ланцюгова реакція довжин рестрикційних фрагментів.

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однонуклеотидних поліморфізма rs2583988 (C> T) в гені альфа-синуклеїна. Текущий поліморфний варіант был выбран путем анализа биоинформатических и генетических исследований. Мы разработали пару праймеров и условия рестрикционного анализа для выявления различных аллелей в rs2583988. Как результат, был разработан простой и доступный метод выявления однонуклеотидных полиморфизма аллелей rs2583988. Ожидается, что метод окажется удобным для клинических лабораторий, благодаря доступной стоимости и оборудованию, а также использоваться в дальнейших ассоциативных исследованиях.

Ключевые слова: болезнь Паркинсона, ген альфа-синуклеїна, однонуклеотидный полиморфизм, полимеразная цепная реакция длин рестрикционных фрагментов.

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FEATURES OF THE ABDUCTION MECHANISM WORK BEFORE AND AFTER TOTAL HIP JOINT ARTHROPLASTY

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Increased tone of the flexors and adductor muscles of the hip in combination with a decrease in elasticity and contractile function of abductors and protractors of the hip due to contracture of the hip joint leads to imbalance in the muscles of the pelvic girdle, which negatively affects the positional adaptation of endoprosthesis components during the surgery and complicates the postoperative period in patients. The treatment results analysis of 114 patients with diseases of the hip joint showed that in most patients the manifestations of pelvic girdle muscle deficiency after hip arthroplasty decreased, but did not disappear for a long time. The study of vertical posture in patients with normal and reduced size of the total femoral offset after hip arthroplasty showed that the reduction of the abductor shoulder leads to a significant change in the main indices of the stabilogram, namely to increased energy expenditure to maintain vertical posture, therefore energy depletion occurs faster. It is clinically manifested by symptoms of pelvic girdle muscle insufficiency - lameness, positive symptom of Trendelenburg, Duchenne, the need to use additional support, etc.

Key words: endoprosthetics, hip joint, vertical posture, statography, femoral offset.

The work is a fragment of the research project "To study the biomechanical features of the standing and walking function in humans after hip arthroplasty", state registration No. 0118U006950.

Owing to the classic works by Yanson Kh.A. [2], Kizilova N. [7] it is generally accepted that maintaining the horizontal balance of the pelvis in the frontal plane is achieved by the work of the pelvic girdle muscles, namely the ability to dynamically balance the gravitational, reactive and muscular forces acting on the joint components in the frontal plane in single-support standby.

The main condition for stable single-support standby is the balance (relative to the center of the hip joint rotation) of the abductor muscles force moments and gravitational and reactive forces, which is determined by the so-called abduction mechanism - the work of pelvic girdle muscles, which provide constant dynamic restoration of the pelvis horizontal balance [11]. To counteract the gravity force, muscles must develop significant efforts. This is especially true of dynamic loads, because in these conditions great effort is required to maintain a horizontal balance of the pelvis. Given that the arm of the abductor muscles is by 2.2-2.5 times shorter than the arm of gravity, to ensure the operation of the abduction mechanism, the muscular effort must exceed the body weight by 1.5 to 3 times [2, 5].

This makes it clear what an important factor in the normal functioning of the hip joint is the functional state of the abductor muscles, i.e. the work of the abduction mechanism of the hip joint to ensure the horizontal balance of the pelvis [4].

The work efficiency of the hip joint is determined by two main factors: the anatomical parameters of the joint elements and the condition of the pelvic girdle muscles, which are closely related. Thus, the change in the anatomical parameters of the hip joint: deformation of the femoral head, its protrusion, elongation of the trochanter major, impairment of the proximal femur torsion, which are caused by various orthopedic diseases and injuries, lead to a change in the force arm of abductor muscles, which adversely affects the abduction mechanism's operation and significantly slows down the process of the patient's

rehabilitation after arthroplasty [1]. In the context of the above, the question arises about the causes and mechanism of the abduction mechanism's dysfunction development (which manifestation is an impairment of the patient's standby) after total hip arthroplasty.

Prolonged pain and contracture of the hip joint lead to contractile spasm and reduction in the length of the adductor and flexor muscles of the hip and to the relative overstretching of the abductor muscles, which leads to a decrease in their elasticity and impaired contractility, and subsequently, to structural muscular changes and significant loss of strength of the abductor muscles.

Reducing the length of the flexors and adductor muscles complicates the installation of the endoprosthesis components, which requires in some cases, the performance of adductors myotomy, excision of the joint capsule. Sometimes excessive soft tissue tension causes the installation of endoprosthesis components with a decrease in the total femoral offset - (distance from the center of the femoral head rotation to the line of the abductor muscles action) [10].

Thus, the working concept of the study can be formulated as follows: the presence of long-term flexion- adductor contracture of the hip joint complicates the positional adaptation of the endoprosthesis components reducing the total femoral offset (arm of abductor muscles), which in its turn negatively affects the abduction mechanism and slows down the process of postoperative rehabilitation in patients.

The purpose of the study was to determine the cause and mechanism of the abduction mechanism dysfunction development and slow down the recovery process after hip joint arthroplasty.

Materials and methods. The study group included 114 patients aged 29 to 76 years (mean age - (56 ± 8) years) with diseases and consequences of the hip joint injuries, who were performed total cementless hip arthroplasty during 2012-2017. The study was performed on the basis of Joint Pathology Clinic and the archive of the Sytenko Institute of Spine and Joint Pathology, NAMS of Ukraine. 47 men (41.2%) and 67 women (58.8%) were examined.

Clinical examination and study of the musculoskeletal system function in patients were performed according to the classical method of the trauma patient's examination.

Functional results of endoprosthetics were assessed in compliance with the Harris rating scale [6], based on clinical trial data and subjective assessment of the patient's condition, the severity of pain was assessed by a visual analog scale (VAS), function of muscles responsible for maintaining postural balance was assessed, according to our improved method [10], the efficacy of maintaining a vertical posture before and after the surgery - using protocol indices of stabilographic study, namely the amplitude and energy parameters of standby for two-support and single-support standing.

The studies comply with the principles and provisions of the Declaration of Helsinki, adopted by the General Assembly of the World Medical Association, on the ethical principles of scientific medical research with human participation (1997-2000).

The study data were processed statistically: descriptive statistics was used with the calculation of the mean (M) and its standard deviation (SD), the range of values. The data analysis for the distribution normality was performed using the Kolmogorov-Smirnov test. Comparisons between groups of patients were performed using the T-test for independent samples. The analysis was performed using the software package IBM SPSS Statistics 20.0. Preliminary data preparation was performed using the Microsoft® Excel® 2013 software package of the Microsoft Office Prof. + 2013.

Results of the study and their discussion. Almost all the patients at the time of consultation complained of pain in the hip joint. At the same time, 39 patients (34.5%) noted moderate pain in the compromised joint, 75 (65.8%) - severe.

After surgical treatment, the severity of the pain changed. The complete absence of pain in the operated joint was noted by 104 patients (91.2%), or they noted mild intermittent pain or discomfort. Nine patients (7.9%) reported moderate, often intermittent pain, which was rated at 3-5 points for hip joints. And only in one case (0.9%) the patient complained of severe pain, which he rated as 7 points according to VAS.

Our particular interest was drawn by clinical indices of postural imbalance (in this case we speak about the abduction mechanism of the hip joint, which provides horizontal balance of the pelvis) in the form of lameness, the use of additional support when walking, symptoms of muscle failure, limited movement and contractures in the hip joint. In table 1 clinical signs of limb dysfunction in patients before surgery and 6 months after surgery are presented.

Thus, after surgery - total hip arthroplasty - the presence of the abduction mechanism insufficiency signs in the form of the pelvis horizontal balance disorder decreased significantly. In particular, the number of patients with pelvic girdle muscles insufficiency, such as lameness and the use of additional support, decreased by approximately 20%, such as the Trendelenburg symptom and the Duchenne symptom - by 50-60%.

Signs of the hip joint abduction mechanism dysfunction, which ensures the horizontal balance of the pelvis, before surgery and 6 months after surgery

Signs of the pelvis horizontal balance disorder	Number of observations (n = 114)	
	Before surgery	6 months after surgery
Lameness	85 (74.6 %)	61 (53.5 %)
Use of additional support	37 (32.5 %)	15 (13.1 %)
Trendelenburg's symptom	103 (90.4%)	49 (43.0 %)
Duchenne's symptom	103 (90.4%)	34 (29.8 %)
Contracture	97 (85.1 %)	7 (6.1 %)

Hip-spine syndrome was detected in 47 patients (41.2%), mainly in patients with bilateral coxarthrosis, and mostly these particular patients had signs of incompetence in muscles responsible for the operation of the abduction mechanism in the post-operative period.

In 97 patients before surgery, adduction-flexion contractures of varying severity were identified, in 58 cases (59.8% of the total number of detected contractures) the magnitude of hip flexion exceeding 20° , and the magnitude of adduction -10° , having clinical manifestations in the form of relative shortening of the diseased limb, pelvic tilt, hyperlordosis, etc. In 28 cases (28.9% of the total number of detected contractures), the amount of hip flexion exceeded 30° , and the amount of adduction -15° .

Thus, it can be stated that in most patients there was a pronounced adduction-flexion contracture of the hip joint, which was clinically manifested by shortening of the limb on the affected side, pelvic tilt to the "healthy" side and forward, hyperlordosis and concomitant lesions, which together formed "pathological" movement strategy.

These clinical indices are usually associated with functional changes in the muscles and determine the musculoskeletal function disorder in the limb and negatively affect the maintenance of postural balance, lead to the development of new motor strategies (because standing is a dynamic type of posture maintaining).

After hip arthroplasty, almost all the patients showed a marked improvement in musculoskeletal function, in addition, over time, most patients showed positive dynamics of improving the function of the muscles responsible for the pelvis horizontal balance.

Functional results of the patients' treatment were assessed according to the standard Harris method. Before the endoprosthesis surgery by Harris, the condition of each patient was assessed in points. The functional status of the hip joint before surgery averaged (32.6 ± 7.1) points. Then the results of treatment for each patient were assessed in points approximately 6 and 12 months after the surgery and, comparing the point scores before the surgery and after the surgery in different terms, determined the dynamics of the recovery process over time.

For further analysis and comparison of treatment results for the study group in total, the mean value and the standard deviation were calculated.

Comparison of mean scores permitted to determine the efficacy of treatment, and comparison of the standard deviation values in the same terms permitted to assess the uniformity of the study group.

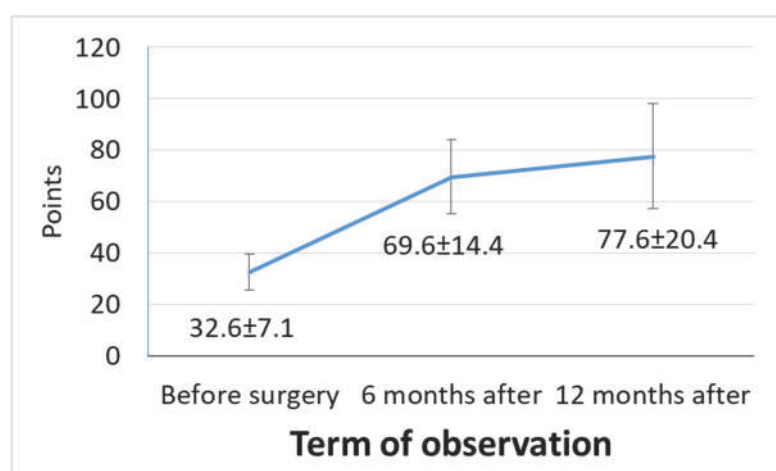


Fig. 1. Diagram of the mean indices' dynamics of the functional state recovery in the operated hip joint and the value of the standard deviation 6 and 12 months after surgery.

The results presented in the diagram (fig. 1) show that after 6 months from the moment of surgery the mean index of functional recovery was (69.6 ± 14.4) points, and up to 12 months. - (77.6 ± 20.4) points.

The mean scores indicate that, on average, in the study group most of the patients had good functional outcome of treatment 6 months after surgery. And over time (after 12 months) the number of patients with good results only increases. For example, 6 months. after surgery, the maximum score by the Harris scale was 84.14 points, and a score exceeding 80 points, which corresponds to an excellent result, only four patients had. After 12 months the maximum score by the Harris scale was 86.54 points, and a score exceeding 80 points was recorded in 19 patients.

It should be noted that the increase in the mean score in the study group was insignificant, and the difference was statistically unreliable. However, the value of the standard deviation has significantly increased. An increase in the standard deviation rate by almost a third reflects a greater variation in treatment outcomes in the group as time passed or a decrease in the group uniformity. This means that 12 months after surgery, most of the patients had excellent results compared to those after 6 months, but this does not mean that in all the patients the results became better 12 months after the operation.

The obtained results of treatment in the patients show that the total hip arthroplasty permits to reliably relieve patients of pain and quickly restore normal household activity. However, muscular incompetence of pelvic girdle occurs even 12 months after surgery. This is evidenced by the presence of lameness, a positive symptom of Trendelenburg and Duchenne signs, which are identified in some patients, i.e. indirect signs of abduction mechanism impairment, which provides horizontal balance of the pelvis and is determined by equality of the gravity force moment and the force moment of hip abductor muscles. The force moment of the hip abductor muscles is determined by the product of the size of the arm of force value by the value of muscle power, which depends on the presence of pain, tone and contractility of the muscle.

However, it is not possible to assess the function of the pelvic girdle muscles using the Harris assessment method, as it focuses on the analysis of the quality of life and the household adaptation of the patient. Therefore, we have proposed and tested a technique based on the principle of maintaining the pelvis horizontal balance while standing and walking due to the muscles of the torso and pelvic girdle.

Details and principles of using this technique are given in the literature [9], so we will only focus on the results obtained.

According to our data, before the surgery all the studied patients had an unsatisfactory condition of the muscles, which are responsible for the work of the abduction mechanism, i.e. for maintaining the horizontal balance of the pelvis. Even conservative treatment of coxarthrosis for 6-12 months does not significantly improve the function of the hip abductor muscles, although the tendency to restore the muscle function after surgery is more pronounced in patients who received such preoperative conservative treatment.

Hip arthroplasty improves the function of the abductor muscles by restoring the geometry of the joint, eliminating (or significantly reducing) the contracture, and relieving the patient of pain. Although in most patients the recovery of muscle function occurs within a year after surgery, but in a number of patients the function of the abductor muscles is never completely restored.

What is the reason for the impairments? We have analyzed the causes of such complications and concluded that it is due to a disorder of the hip joint abduction mechanism, which is determined by two indices - the arm of force length and the maximum power of the abductor muscles. The product of these parameters determines the magnitude of the abductor muscles' force moment, which counteracts the moment of gravity. Given this, a very important index is the size of the abductor muscles' arm of force, and the condition of the muscles before and after surgery.

The magnitude of the hip abductor muscles' arm of force after arthroplasty is determined by an index called the total femoral offset. If the value of the total femoral offset after hip arthroplasty is equal to the length of of a healthy joint abductors' arm of force, as it is the case in most patients, the restoration of muscle function occurs in a short time. But in more than 30% of patients according to our data [7, 11] the value of the total femoral offset decreases after surgery.

We have studied the effect of this factor on muscle function by means of stabilographic studies, which are objective evidence of the abduction mechanism dysfunction in clinical settings [11].

According to the archival materials of the laboratory of biomechanics at the SI "Sytenko Institute of Spine and Joint Pathology, NAMS of Ukraine" there were selected stabilogram protocols of 30 patients with coxarthrosis before and after hip arthroplasty, of which 15 had the total femoral offset equal to the arm of force length of abductors on the opposite side, and 15 patients had a lower total femoral offset, according to measurements in radiographs.

According to the comparative statographic analysis, there were some differences in the standing characteristics of the patients in whom the size of the abductor muscles' arm after arthroplasty reduced compared to similar indices of the patients in whom changes in the abductor muscle arm's length did not occur. It has been established that after arthroplasty reduction of the abductor muscle arm's length leads to a significant change in the shape and size of the center of mass projection (during single support of standby), namely: to its sagittal size increase and the frontal size reduction in the center of mass displacement projection spot, which indicates a greater amplitude of body oscillations in the sagittal plane. It was also found that reduction in the length of the abductor muscles arm after arthroplasty leads to an increase in the speed and frequency of oscillations in the center of mass projection (table 2).

Under these conditions, energy expenditures significantly (more than by 2 times) increase to maintain a vertical posture. Thus, energy depletion occurs more quickly, which is clinically manifested by the symptoms of pelvic girdle muscle incompetence - lameness, a positive symptom of Trendelenburg, Duchenne, the need to use additional support, etc.

Analysis of statograms spectral characteristics in patients with reduced (group I) and unchanged (group II) total femoral offset 9-12 months after hip joint arthroplasty

Test	Group		Before treatment				After treatment			
			F(Hz) (min÷max)	ΣF(Hz) (Mo)	P(W) (min÷max)	ΣP(W) (M, SD)	F(Hz) (min÷max)	ΣF(Hz) (Mo)	P(W) (min÷max)	ΣP W) (M, SD)
double-support	I n=15	X1	0.1÷0.8	0.2	50÷250	464±101	0.1÷0.5	0.2	25÷75	129±46
		Y1	0.1÷1.1		130÷690		0.13÷0.7		60÷200	
	II n=15	X1	0.1÷1.0	0.2	50÷250	544±104	0.1÷1.0	0.3	20÷50	269±24
		Y1	0.1÷0.9		200÷480		0.2÷0.5		200÷280	
T-test (t, p)		x				t=-1.744 p=0.098	x		t=-8.570 p=0.001	
Injured limb	I n=15	X1	0.1÷0.5	0.5	250÷550	1013±238	0.12÷1.1	0.7	240÷270	1122±29
		Y1	0.1÷1.0		400÷1000		0.2÷1.1		800÷1000	
	II n=15	X1	0.2÷0.6	0.4	180÷240	1041±271	0.2÷0.6	0.5	150÷175	494±10
		Y1	0.1÷0.7		380÷1040		0.1÷0.7		320÷350	
T-test (t, p)		x				t=-0.244 p=0.810	x		t=65.135 p=0.001	
Conditionally healthy limbs	I n=15	X1	0.12÷0.6	0.3	30÷70	150±57	0.1÷0.8	0.3	70÷90	288±16
		Y1	0.3÷0.6		50÷150		0.04÷0.4		160÷220	
	II n=15	X1	0.2÷0.9	0.2	45÷80	145±65	0.2÷0.9	0.3	50÷70	230±10
		Y1	0.3÷0.9		130÷160		0.3÷0.9		100÷240	
T-test (t, p)		x				t=0.169 p=0.867	x		t=3.714 p=0.065	

Hip joint arthroplasty in the long-term course of coxarthrosis permits to effectively relieve the patient of pain, to restore range of motion, significantly improve the quality of life in patients. But there is a study which says about reduction of the hip joint's support and kinematic function, which is observed for a long time after surgery. In particular, according to the performed meta-analysis of the literature, Vissers et al. [12] showed that the strength of the abductors is not restored in the next 6 months after hip arthroplasty. Similar conclusions were made by other authors [3], who studied the postural stability of patients after the above surgeries.

Unfortunately, researchers focus on the muscles condition when assessing the stability of standing in the postoperative period, but almost do not take into account the condition of the muscles before surgery. Experts who study the support and kinematic function of the extremities after hip arthroplasty believe that insufficiently rapid recovery of muscle activity and soft tissue tone is the result of inadequate restorative treatment in the postoperative period [8]. We suggested that the hip joint contracture, in the long-term course of coxarthrosis is one of the important factors influencing the work of the hip joint's abduction mechanism. According to our data, the vast majority of patients with coxarthrosis - more than 85% - had hip contractures, and most of them (almost 60% of all patients with contractures) had a severe degree of contractures.

In our opinion, the presence of flexion-adduction contracture, which is accompanied by retraction of the adductive muscles and flexors and relative overstretching of the abductors with a decrease in their tone and contractility, has a significant impact on positional adaptation of implant components during surgery, namely in more than 30% of patients it reduces the size of the total femoral offset compared to the arm of force length in a healthy joint's abductors.

In recent years, works have begun to appear raising the question of the total femoral offset size effect on the hip abductor muscles strength [9]. However, the authors have only analyzed the muscle strength indices mainly of the operated limb, but did not study the effect of the total femoral offset on the work of the abduction mechanism, namely on the maintenance of the pelvis horizontal balance. Our stabilographic studies have shown that reduction in the size of the total femoral offset leads to certain noticeable impairments of the vertical posture, in particular, an increase in energy expenditure to maintain a vertical posture by more than 2 times. And this in its turn leads to energy depletion of muscles and slows down the process of postoperative rehabilitation in patients.

Conclusions

1. Contracture of the hip joint in the prolonged course of coxarthrosis negatively affects the positional adaptation of the endoprosthesis components, namely, creates conditions for reducing the size of the total femoral offset.

2. Reduction of the total femoral offset in its turn is a negative factor influencing the hip joint abduction mechanism, namely the horizontal balance of the pelvis, which has clinical manifestations in the form of lameness, lateral tilt of the pelvis and torso, etc. in the postoperative period.

3. Stabilographic studies have objectively proven that reduction of the total femoral offset size has a negative impact on the hip joint abduction mechanism, and slows down the recovery process after hip joint arthroplasty due to increased energy expenditures for maintaining a vertical posture.

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Реферати

ОСОБЛИВОСТІ РОБОТИ АБДУКЦІЙНОГО МЕХАНІЗМУ ДО ТА ПІСЛЯ ТОТАЛЬНОГО ЕНДОПРОТЕЗУВАННЯ КУЛЬШОВОГО СУГЛОБА

Тяжелов О.А., Карпінська О.Д., Гончарова Л.Д., Климовицький Ф.В.

Підвищення тонусу згиначів і привідних м'язів стегна у поєднанні зі зменшенням еластичності та скорочувальної функції абдукторів та розгиначів стегна внаслідок контрактури кульшового суглоба призводить до виникнення дисбалансу у м'язах тазового пояса, що негативно позначається на позиційній адаптації компонентів ендопротезу під час операції та ускладнює післяопераційний період у хворих. Аналіз результатів лікування 114 пацієнтів із захворюваннями кульшового суглоба, показав, що більшості пацієнтів прояви дефіциту м'язів тазового пояса після ендопротезування зменшилися, але не зникли тривалий час. Вивчення вертикальної пози у пацієнтів із нормальним та зменшеним розміром загального стегнового офсету після ендопротезування кульшового суглоба показало, що зменшення плеча абдукторів призводить до значної зміни основних показників стабілограми, а саме до збільшення енергетичних витрат на підтримку вертикальної постави, тому виснаження енергії відбувається швидше, це клінічно проявляється симптомами недостатності м'язів тазового дна - кульгавістю, позитивним симптомом Тренделенбурга, Дюшенна, необхідністю використання додаткової підтримки тощо.

Ключові слова: Ендопротезування, кульшовий суглоб, вертикальна поза, статографія, стегновий офсет
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ОСОБЕННОСТИ РАБОТЫ АБДУКЦИОННОГО МЕХАНИЗМА ДО И ПОСЛЕ ТОТАЛЬНОГО ЭНДОПРОТЕЗИРОВАНИЯ ТАЗОБЕДРЕННОГО СУСТАВА

Тяжелов А.А., Карпинская Е.Д., Гончарова Л.Д., Климовицкий Ф.В.

Повышение тонуса сгибателей и приводящих мышц бедра в сочетании с уменьшением эластичности и сократительной функции абдукторов и разгибателей бедра вследствие контрактуры тазобедренного сустава приводит к возникновению дисбаланса в мышцах тазового пояса, что отрицательно сказывается на позиционной адаптации компонентов эндопротеза во время операции и затрудняет послеоперационный период у больных. Анализ результатов лечения 114 пациентов с заболеваниями тазобедренного сустава показал, что у большинства пациентов проявления дефицита мышц тазового пояса после эндопротезирования уменьшились, но не исчезали длительное время. Изучение вертикальной позы у пациентов с нормальным и уменьшенным размером общего бедренного офсета после эндопротезирования тазобедренного сустава показало, что уменьшение плеча абдукторов приводит к значительному изменению основных показателей стабилограммы, а именно к увеличению энергетических затрат на поддержание вертикальной осанки, поэтому истощение энергии происходит быстрее, это клинически проявляется симптомами недостаточности мышц тазового дна - хромотой, положительным симптомом Тренделенбурга, Дюшенна, необходимостью использования дополнительной поддержки и тому подобное.

Ключевые слова: эндопротезирование, тазобедренный сустав, вертикальная поза, статография, бедренный офсет.

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ASSESSMENT OF RESPIRATORY SYSTEM'S FUNCTIONAL STATE IN CHILDREN WITH BRONCHIAL ASTHMA AND ALLERGIC RHINITIS

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The study of the external respiration function by the method of spirometry was performed in 138 children from 7 to 17 years of age, among them, observation group I included 78 children with bronchial asthma; group II included 40 children with allergic rhinitis; group III included 20 virtually healthy children. It was established that in children with bronchial asthma, the obstructive pulmonary ventilation function was impaired in 67.95% of cases, the restrictive one - in 16.67% and the mixed one - in 2.56% of patients. In children with allergic rhinitis, the obstructive ventilation disorders in 32.5% of cases were caused by the combined orthodontic pathology, namely, the distal occlusion and in 12.5% - by chronic allergic inflammation of the respiratory tract without tooth-jaw anomalies, which required an individual approach to the choice of treatment and prevention. The functional state indices of the respiratory system during spirometry should be taken into account both for determining the type of ventilation disorders in children with bronchial asthma and for differential diagnostics of chronic allergic inflammation of the respiratory tract and associated orthodontic pathology in children with allergic rhinitis.

Key words: bronchial asthma, allergic rhinitis, children, spirometry.

The work is a fragment of the scientific project "Optimization of differential diagnostics and treatment of allergic and other diseases in children of different ages", state registration No. 0118U004254.

At present, allergic diseases unceasingly continue to spread throughout the world with the incidence of bronchial asthma in the population ranging from 1% to 18% and allergic rhinitis – from 10% to 40%. [13] According to the modern concept of "single chronic allergic respiratory syndrome", allergic rhinitis and bronchial asthma have common triggers and pathogenetic mechanisms for the development of an immediate-type allergic reaction [7]. Although there is no "gold standard" for diagnostics of bronchial asthma (BA), the diagnostics criteria for this disease are the presence of both respiratory symptoms and the definition of expiratory disorders according to spirometry data: the decrease of the forced expiration volume indices in the first second (FEV₁) and the ratio of the forced expiration volume in the first second to the function of vital capacity of the lungs (FEV₁/VC). [6] At the same time, the diagnostics of allergic rhinitis is based on clinical symptoms such as sneezing, itching, nasal congestion, discharge and loss of sensory sensitivity that deteriorate the quality of life in these patients even worse than in patients with mild or even with moderate severe bronchial asthma [3]. Impairment of the nasal breath also affects other functions of the organism, in particular the respiratory system, contributing to the development of external respiration dysfunction - restriction of chest excursion when the breathing becomes frequent and superficial, which results in pulmonary ventilation decreasing. Also, in an allergic rhinitis, the child constantly breathes through its open mouth, and the associated tension of the face muscles and the change in the configuration and growth of the face bones, head, upper and lower jaws are one of the leading etiological factors in the formation of distal occlusion in children and reduction of lungs life volume and impaired breathing, due to mechanical obstruction for normal breathing [11]. Today, in the field of practical medicine, the study of nasal breathing function (rhinopneumometry) has not been widely used, but there are works showing the diagnostics value of the study on the respiratory system's functional state in children with allergic rhinitis or with orthodontic pathology [1]. However, the nature of ventilatory disorders in children, both in case of chronic allergic inflammation and in case of orthodontic pathology, has not been completely studied. All this determines the relevance of the chosen research subject.

The purpose of the study was assessment of the lungs' ventilation function and performing the comparative analysis of the external respiration parameters in children with bronchial asthma and allergic rhinitis.

Materials and methods. In total 138 children aged 7 to 17 years old (mean age 13.5±2.84, namely 50 girls (36.23%) and 88 (63.77%) boys, were examined. Among them, group I consisted of 78 children with bronchial asthma (27 girls (34.62%) and 51 boys (65.38%); group II – 40 children with allergic rhinitis (14 girls (35%) and 26 boys (65%); group III (control) – 20 virtually healthy children (9 girls (45%) and 11 boys (55%). Although there were more boys than girls, however, the ratio of boys and girls in each group, when compared, did not have statistically significant differences, p>0.05. The function of external respiration was studied by spirometry method using a computer complex with an adapted software for the study of external respiration function "PULMOREM" TU U 33.1-02066769-005-2002 (Kharkiv) with automatic analysis of indices. The maneuver of forced expiration was performed three times, after which

the types of ventilation disorders were determined according to the main indices that characterized the function of external respiration: static lungs volume – vital capacity of the lungs (VC) and dynamic lungs volumes - forced vital capacity of the lungs (FVC), the forced expiration volume per one second (FEV1) and the ratio of FEV1/FVC%). Also, the parameters such as the maximal expiratory flow at the level of 25%, 50% and 75% of FVC (MEF25, MEF50 and MEF75) were analyzed. In order to diagnose the hidden bronchospasm, the children were provided with a broncholytic test with inhalation of 200 µg or 400 µg (depending on the age) of salbutamol, which was considered to be positive if the gain of FEV1 occurred to be equal to or exceeded 12% (or >200 ml). The statistical data processing was performed by means of commonly used methods of variation statistics using the licensed software package Statistica for Windows 6.1.RU, serial number AXXR712D833214SAN5. The non-parametric statistical methods were used: the medians and interquartile intervals were calculated, and the two independent groups were compared according to the Mann-Whitney criterion, the criterion χ^2 , and “2×2 Table”. At $p < 0.05$, the differences were considered statistically significant.

Results of the study and their discussion. In the result of the spirometric study, it was found that the medians and interquartile intervals of the most external respiration function indices in children, both with bronchial asthma and allergic rhinitis, were significantly different from those of healthy children and between each other (table 1).

Table 1

Indices of the external respiration function in children (Me (Q25–Q75))

Groups	I	II	III
VC (%)	65.91 (56.83–79.19) *II,*III	94.00 (81.05–100.48) *I,*III	107.00 (100.48–111.00) *I,*II
FVC(%)	75.13 (63.73–87.05) *II,*III	93.00 (75.79–101.5) *I	99.05 (93.1–100.25) *I
FEV ₁ (%)	73.97 (62.57–81.84) *II,*III	92.5 (70.52–104.3) *I,*III	105.15 (100.25–108.15) *I,*II
FEV ₁ / FVC(%)	93.84 (81.12–100) *II,*III	98.32 (85.86–106.5) *I,*III	107.20 (102.95–110.65) *I,*II
MEF ₂₅ (%)	75.89 (64.56–90.54) *II,*III	95 (68.54–102.65) *I,*III	101.6 (94.9–117.5) *I,*II
MEF ₅₀ (%)	82.45 (69.8–103.85) *III	93.5 (70.88–105.05)	96.6 (77.75–102.7) *I
MEF ₇₅ (%)	82.01 (56.5–114.02) *II,*III	99.0 (64.99–122.94) *I	95.8 (86.4–109.5) *I

Note: *I, II, III - reliability of the difference between the groups of children ($p < 0.05$).

So, certainly, the highest results of the static index of VC and such dynamic indices as FEV1, FEV1/FVC, MEF 25 were registered in healthy children, and the lowest – in children with bronchial asthma. However, contrary to the anticipated data, in children with allergic rhinitis, although the medians of these data were significantly higher than the results of children with bronchial asthma, but did not reach the values of the healthy ones. The group of children with bronchial asthma had significantly lower FVC indicators and maximal expiratory flow at the level of 75 % or 50 % of FVC volume remaining in the lungs: MEF75 and MEF50, which was reflected by the resistance of respiratory tracts at a level of 75% of FVC of brochi with a diameter less than 3 mm, that is, small bronchi, and 50 % of bronchi FVC of medium caliber. Consequently, there was a uniform decrease in all indices of MEF 25 % – 75 % only in children with bronchial asthma, indicating a total (generalized) type of bronchial obstruction.

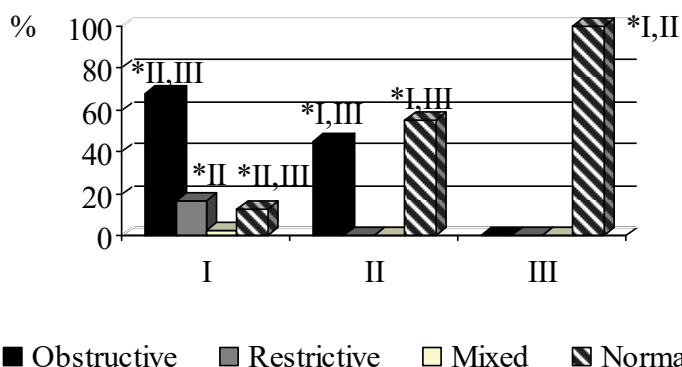


Fig. 1. Characteristics of the types of ventilation function of the lungs in children.

The generalized results of the study of volumetric and high-flow indicators of the function of external respiration and types of ventilatory disorders of the lungs in children are presented in the fig. 1.

It should be immediately noted that all healthy children (group III) had no disorders of the lungs ventilation function. However, in children of group I with bronchial asthma, the lungs ventilation function was impaired in 87.18% (68/78) and normal - only in

12.82 % (10/78) cases, and in children, in which the changes in the parameters of external respiration function indices were not found at once, the pharmacological test was performed with a short-acting beta-agonist, and, only in the absence of hidden bronchospasm, the ventilation function of the lungs was considered as normal one.

In children with allergic rhinitis (observation group II), the ventilation disorders were registered in 45% (18/40) patients, and in 55% (22/40) cases the function of external respiration was normal. At the same time, the obstructive ventilation disorders were registered in 67.95 % (53/78) of children with bronchial asthma versus 45 % (18/40) in children with allergic rhinitis ($\chi^2 = 12.13$, $p = .0005$ – between the comparison groups I and II; $\chi^2 = 31.14$, $p = .0000$ – between groups I and III, $\chi^2 = 12.86$, $p = .0003$ – between groups II and III). The restrictive type of ventilation disorders was found only in 16.67 % (13/78) of children with bronchial asthma ($\chi^2 = 7.49$, $p = .0062$ – between groups I and II and $\chi^2 = 4.49$, $p = .0342$ – between children groups I and III). The mixed type of ventilation disorders was recorded in 2.56% (2/78) of the examined persons from group I. The restrictive and mixed types of external respiration functional disorders in children with bronchial asthma were caused by the presence of uncontrolled severe course of the disease. The obstructive changes of the spirometry indices of the examined children indicated that they had an obstacle to the air flow. In children with bronchial asthma, the obstructive ventilation disorders were caused by the hyper-reactivity of the bronchi, bronchial spasm, inflammation of the bronchial mucosa and edema. In order to determine the cause of ventilation disorders in children with allergic rhinitis, all children from group II were examined by a pediatric orthodontist for the diagnostics of tooth-jaw pathology, as an additional mechanical obstacle for normal breathing. Thus, it was found that 32.5% (13/40) of children with allergic rhinitis and obstructive type of ventilation disorders also had an orthodontic pathology in the form of distal occlusion, which became an additional mechanical obstacle when performing respiratory maneuver in these children during the spirometric study. These children subsequently received treatment from an allergist and pediatric orthodontist. Other 12.5 % (5/40) of children with allergic rhinitis and obstructive type of ventilation disorders, according to the modern concept, had chronic allergic inflammation of the respiratory tract, but without combination with orthodontic pathology, and also needed an individual approach in the appointment of therapeutic and preventive measures.

In the course of the discussion we compared our results with the data presented in the reference list and stated that when assessing the function of external respiration in children, it was necessary to consider both the main bronchopulmonary pathology and concomitant diseases, including those related to the dentofacial system. Thus, it was the spirometry used by Y.I. Feshchenko et al. for the implementation of the personified approach to confirmation of diagnosis with combined pathology of bronchial asthma and chronic obstructive pulmonary disease in adult patients with bronchopulmonary pathology with FEV/FVC ratio less than 70% [10].

However, in our study in children with bronchial asthma, the median rates of FEV / FVC ratio on the contrary exceeded 70% and amounted to 73.97 %, which indicates better functional state of the respiratory system in childhood without concomitant chronic obstructive pulmonary disease, when fixed wheezing and fixed hyperinflation are still not formed. At the same time, in children with acute bronchopulmonary diseases such as bronchitis, pneumonia, pleurisy, the mean VC was significantly reduced and was within the range of $59,94 \pm 18,71$ % close to the median rates of the children with bronchial asthma examined by us ($Me = 65.91$ (56.83 – 79.19)). Significance of forced vital capacity of the lungs (FVC) and volume of forced exudation for 1 sec. with FEV1 (FEV1) in acute bronchopulmonary diseases (58.98 ± 17.86 % and 57.32 ± 19.51 %) were lower than the median in children with bronchial asthma in our study (75.13 % (63.73 – 87.05) and 73.97 % (62.57 – 81.84)), however, out of 143 children with acute bronchopulmonary disease, restrictive disturbances were noted in 123 children, while the obstructive type in children with bronchial asthma was more commonly recorded (67.95 %) [5]. The study of the external respiration function, carried out in preschool children with wheezing syndrome, permitted to establish a diagnosis of bronchial asthma in 48.3% of examined patients [9].

The individual approach to the determination and assessment of reverse wheezing in children with bronchial asthma and more than 80% of the appropriate FEV1 (FEV1) values, regardless of the disease severity and the symptom control level, was scientifically substantiated by Rechkina O.O. et al. [8]. Comparative characteristics of the external respiration function in children with bronchial asthma and allergic rhinitis are presented in the work of Nevine El-Helaly et al. Thus, in children with bronchial asthma, the outgoing mean FVC rates were higher than in our study and amounted to 91.215 versus 75.13 in our study; FEV1 was 96.875 vs. 73.97. In patients with allergic rhinitis, the FVC and FEV1 data were identical to ours: 94.32 and 93 and 96.81 and 96.81, respectively [14].

However, in the work of Saranz R.J. et al., according to the results of the study, the spirometric parameters were reported in 22 % of children with allergic rhinitis, while in our study the ventilation

abnormalities were almost twice as frequent (45 %). [15]. The need for an objective study of the lungs' ventilation function in children with bronchial asthma is evidenced by the work of Alexander Moeller, who believes that the spirometric study should be periodically performed not only in children with bronchial asthma, but also in those with allergic rhinitis, especially if they have a risk of sleep-related breathing disorder [12].

As well as in our study in patients with distal occlusion, the reduction of the rates of external respiration was established in children with cutter disocclusion of dentition [4]. And the study of A.A. Adamchik showed that in children with anomalies of bite, the mild and moderate bronchial asthma disorders, which were not accompanied by clinical manifestations, were recorded in 84 % of cases due to lower FEV₁, PIF, maximum bulk volume velocity at the 75%-level of forced lung capacity volume (MEF75) remaining in the lungs and Tiffeneau's index. At the same time, the decrease of VC in the range of 70-73 % was noted in 16 % of the examined patients with orthodontic pathology [1]. It was specified that in children with open bite and myofunctional disorders, the decrease in the parameters of external respiration was also recorded, including VC, which was decreased by 30.7 % compared to the control group without myofunctional violations [2].

The given data confirm the necessity of studying the function of external respiration during the examination of children both with bronchial asthma and with allergic rhinitis and orthodontic pathology.

Conclusions

1. The lungs' ventilation function in children with bronchial asthma in 67.95 % was disordered according to the obstructive type with a generalized decrease of the MEF indices by 25 % -75 %, the restrictive (16.67 %) and mixed types (2.56 %) were due to an uncontrolled severe course of the disease.

2. In children with allergic rhinitis, the obstructive ventilation disorders in 32.5 % of cases were caused by the combined orthodontic pathology, which complicated the implementation of respiratory maneuvers and in 12.5% by the presence of "single chronic allergic respiratory syndrome" without tooth-jaw anomalies, which required an individual approach in the prescription of medical and preventive measures.

3. The respiratory system's functional state indices during spirometry should be taken into account both for determining the type of ventilation disorders in children with bronchial asthma and for differential diagnostics of chronic allergic inflammation of the respiratory tract and concomitant orthodontic pathology in children with allergic rhinitis.

Prospects for further research. In the future, it is planned to give a comparative characteristics of indicators of external respiration in groups of children with different degrees of severity and controllability of bronchial asthma and in groups of patients only with allergic rhinitis, only with orthodontic pathology (with distal occlusion) and with a combination of allergic rhinitis and distal occlusion defining the most informative indicators for early diagnostics of ventilation disorders and a differentiated approach to prediction, prevention and treatment of these states in children.

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Реферати

**ОЦІНКА ФУНКЦІОНАЛЬНОГО СТАНУ
ДИХАЛЬНОЇ СИСТЕМИ У ДІТЕЙ
З БРОНХІАЛЬНОЮ АСТМОЮ ТА АЛЕРГІЧНИМ
РИНИТОМ**

Шумна Т.Є., Федосєєва О. С., Зінченко Т. П.

Дослідження функції зовнішнього дихання методом спірометрії проводилося у 138 дітей від 7 до 17 років, з них, I групу спостереження склали 78 дітей з бронхіальною астмою; II групу - 40 дітей з алергічним ринітом; III групу - 20 практично здорових дітей. Встановлено, що у дітей з бронхіальною астмою, вентиляційна функція легенів за обструктивного типу була порушена в 67,95% випадків, за рестриктивного - в 16,67% і за мішаного - у 2,56% пацієнтів. У дітей з алергічним ринітом обструктивні вентиляційні порушення в 32,5% випадків були обумовлені наявністю поєднаної ортодонтичної патології, а саме, дистальним прикусом і в 12,5% - наявністю хронічного алергічного запалення респіраторного тракту без зубо-щелепних аномалій, що вимагало індивідуального підходу у виборі лікування і профілактики. Спірометрію необхідно проводити всім дітям з клінічними симптомами респіраторної алергії, а показники функціонального стану дихальної системи необхідно враховувати як для визначення типу вентиляційних порушень у дітей з бронхіальною астмою, так і для диференціальної діагностики хронічного алергічного запалення респіраторного тракту і супутньої ортодонтичної патології у дітей з алергічним ринітом.

Ключові слова: бронхіальна астма, алергічний риніт, діти, спірометрія.

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**ОЦЕНКА ФУНКЦИОНАЛЬНОГО СОСТОЯНИЯ
ДЫХАТЕЛЬНОЙ СИСТЕМЫ У ДЕТЕЙ
С БРОНХИАЛЬНОЙ АСТМОЙ И АЛЛЕРГИЧЕСКИМ
РИНИТОМ**

Шумная Т.Е., Федосеева Е. С., Зинченко Т. П.

Исследование функции внешнего дыхания методом спирометрии проводилось у 138 детей от 7 до 17 лет, из них, I группу наблюдения составило 78 детей с бронхиальной астмой; II группу - 40 детей с аллергическим ринитом; III группу - 20 практически здоровых детей. Установлено, что у детей с бронхиальной астмой, вентиляционная функция легких по обструктивному типу была нарушена в 67,95% случаев, по рестриктивному - в 16,67% и по смешаному - у 2,56% пациентов. У детей с аллергическим ринитом обструктивные вентиляционные нарушения в 32,5% случаев были обусловлены наличием сочетанной ортодонтической патологией, а именно, дистальным прикусом и в 12,5% - наличием хронического аллергического воспаления респираторного тракта без зубо-челюстных аномалий, что требовало индивидуального подхода в выборе лечения и профилактики. Спирометрию необходимо проводить всем детям с клиническими симптомами респираторной аллергии, а показатели функционального состояния дыхательной системы необходимо учитывать как для определения типа вентиляционных нарушений у детей с бронхиальной астмой, так и для дифференциальной диагностики хронического аллергического воспаления респираторного тракта и сопутствующей ортодонтической патологии у детей с аллергическим ринитом.

Ключевые слова: бронхиальная астма, аллергический ринит, дети, спирометрия.

Рецензент Похилько В.І.

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**GLUCOCORTICOIDS AS IMMUNOSTIMULATORS IN PATHOGENETIC THERAPY
OF TUBERCULOSIS**

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The immunostimulating effect of corticosteroids in the complex treatment of tuberculosis patients when they are administered in a double physiological dose, every other day, taking into account the circadian rhythm of the function of the hypothalamic-pituitary-adrenal axis was substantiated in the article. Whereas with daily administration, corticosteroids have shown an immunosuppressive effect. Peripheral blood B-lymphocytes are not sensitive to glucocorticosteroid drugs.

Key words: tuberculosis, pathogenetic therapy, glucocorticosteroids.

This work is a fragment of the research project "To study the effectiveness of pathogenetic agents in the complex treatment of destructive pulmonary tuberculosis with resistance to antibacterial drugs", state registration No. 0117U000304.

Tuberculosis (TB) disease is the result of the organism's immune response to infection with Mycobacterium tuberculosis (Mtb), but becoming infected with Mtb does not mean being sick. According to data of the World Health Organization, no more than 10% of infected people become ill during their lifetime, which indicates the high effectiveness of the human body's defense system. The main condition for tuberculosis development is immunodeficiency disease. The modern medical guideline for tuberculosis

treatment involves only antibacterial drugs, and such a resource as pathogenetic therapy, aimed at restoring the protective capacity of the patient's organism, remains unused.

An important means of pathogenetic therapy of tuberculosis are glucocorticoid (GCs) medications, in which product label tuberculosis is on the list of contraindications due to their immunosuppressive effect. Because of the wide range of biological activity, anti-inflammatory and desensitizing action, GCs are still used in the treatment of patients with tuberculosis [3, 5, 7].

The regulatory effect of GCs on lymphocytes is characterized by a circadian rhythm, while in the morning lymphocytes are more resistant to the cytotoxic effects of GCs, and in the evening their sensitivity increases [6]. The circadian rhythm of quantitative changes in subpopulations of lymphocytes and cortisol shows a high correlation. In physiological concentrations, cortisol is a necessary factor in the formation of the inductive phase of the immunological response [2, 10]. Moreover, in high doses, GCs suppress, and in low doses they increase a resistance to infections of experimental animals. High doses of GCs can even show cytolytic activities on lymphocytes [6]. That is why in the early period the use of GCs in the treatment of tuberculosis and non-specific diseases was accompanied by the development of "steroid" tuberculosis [1]. Similar reports have contributed to limit the use of GCs in the treatment of patients with tuberculosis. Prescribing them to such patients was considered illogical and harmful, since immunodeficiency is the main condition for the development of tuberculosis.

Given the results of scientific studies on the circadian rhythm of quantitative changes in GCs and lymphocytes, we have developed a method of hormone therapy for patients with tuberculosis with the administration of corticosteroids every other day, taking into account the circadian rhythm of the hypothalamic-pituitary-adrenal (HPA) axis. As a result, their negative impact on the immune system was leveled, which made it possible to carry out the entire course of hormone therapy at a dose of 20-30 mg of prednisolone, lasting at least 2 months and to withdraw it simultaneously without tapering the dose. Complications and immunosuppression with this method of hormone therapy are absent.

The purpose of the study was to substantiate the immunostimulatory effect of corticosteroids when administered in a double physiological dose, every other day, taking into account the circadian rhythm of HPA axis functioning in the conditions of complex treatment of patients with tuberculosis.

Materials and methods. The effectiveness of treatment of 304 patients with pulmonary tuberculosis was studied. 134 of them were in the 1st group: received only anti-tuberculosis chemotherapeutics. The 2nd group consisted of 67 patients who received chemotherapy and prednisolone daily in three doses with gradual dose reduction before GCs withdrawal. The 3rd group consisted of 103 patients who received chemotherapy and prednisolone according to the proposed method, with simultaneous GCs withdrawal, without dose tapering.

The functional state of the immune system was determined in 141 patients aged 19-65 years (mean age was 35.9 ± 1.3 years), there were 38 women (27.0%), 103 (73.0%) men. Distribution by clinical forms of pulmonary tuberculosis: in 34 (24.1%) it was focal, in 45 (31.9%) – infiltrative, in 48 (34.0%) – disseminated and in 14 (10.0%) – fibrous–cavernous one. In 108 (76.6%) patients the tuberculous process was destructive and bacteriologically proven.

Overall quantitative indices of cellular, humoral, and phagocytic immunity were determined. The functional state of T-lymphocytes was determined by skin sensitivity to tuberculin with PPD 2 T.U. The humoral immunity was determined by the number of circulating B-lymphocytes, and their functional activity by quantitative changes in plasma immunoglobulins A, M, G. Phagocytic system state was determined by phagocytic activity and phagocytic index of polymorphonuclear neutrophils (PMNs) and mononuclear phagocytic system (MPS).

Examinations were performed at hospitalization of patients, after 2-3 months and at the end of standard treatment guidelines. In accordance with the purpose of the study, we carried out an analysis of the results obtained in 2-3 months, when the intensive phase of chemotherapy of patients with tuberculosis was completed. This made it possible to make a comparative assessment of the effect of GCs on the immune system when administered daily in 3 doses and once, in the morning, every other day.

The selection and examination of patients complied with the principles of biomedical ethics. Study results were analyzed with an Excel computer package for Windows XP, the difference was considered statistically significant at $p < 0.05$.

Results of the study and their discussion. Results of the study showed that, depending on the treatment regimen, GCs had different effects on the immune system of patients. Thus, after 2-3 months of treatment, the total count of lymphocytes in patients of Group 1 who received only anti-TB chemotherapy, was 2.02 ± 0.12 g/l, which corresponded to the norm. The situation was similar in patients of the 3rd group who received GCs every other day, in the intermittent regimen. In them, this index increased to the normal

level from 1.65 ± 0.08 G/l to 2.03 ± 0.07 G/l ($p < 0.01$). And only in the group of patients who received GCs daily in three doses, the level of lymphocytes remained at the initial value level (1.49 ± 0.10 G/l) and amounted to 1.64 ± 0.12 G/l ($p > 0.05$).

T-lymphocytes count was characterized by ambivalent indices. Thus, in patients of the 1st group after 2-3 months of treatment, their number was 0.96 ± 0.11 G/l with a tendency to increase (0.74 ± 0.12 G/l, $p > 0.05$). In patients of the 2nd group, their number decreased to 0.61 ± 0.05 G/l compared with the initial level (0.79 ± 0.08 , $p > 0.05$). And only in patients of the 3rd group T-lymphocytes' count increased to normal value and amounted to 1.21 ± 0.04 G/l, which significantly exceeded the initial level (0.71 ± 0.05 , $p < 0.01$). Correlation analysis of the results confirmed the relationship between changes in the number of lymphocytes and their T-subpopulation with the administration regimen of corticosteroids ($r = 0.515$). The results confirmed that GCs with daily administration showed an immunosuppressive effect on the T-cell immune system, whereas with intermittent administration, their action was characterized by an immunostimulatory effect with the T-cell immunity normalization by the end of hormone therapy.

Humoral immunity was less sensitive to GCs administration. Thus, the number of B-lymphocytes in the peripheral blood of patients with tuberculosis averaged 0.30 ± 0.03 G/l, which reflected a tendency to increase compared to healthy patients (0.23 ± 0.03 G/l, $p > 0.05$). After 2-3 months of treatment, the number of B-lymphocytes was characterized by a tendency to decrease in patients of the 1st group from 0.30 ± 0.03 G/l to 0.25 ± 0.03 G/l ($p > 0.05$), in the 2nd group – from 0.30 ± 0.02 G/l to 0.27 ± 0.02 G/l ($p > 0.05$) and in the 3rd group – from 0.31 ± 0.03 G/l up to 0.24 ± 0.002 G/l ($p > 0.05$), approaching the normal range.

Functional activity of B-lymphocytes by determining the amount of serum immunoglobulins also changed little. In patients with tuberculosis, a significant increase was found only in IgG, and averaged 12.53 ± 0.37 G/l, which exceeded the normal range of 10.21 ± 0.21 G/l ($p < 0.001$). IgA and IgM were 2.33 ± 0.15 G/l and 1.14 ± 0.12 G/l, respectively, which significantly exceeded the corresponding control values (2.10 ± 0.14 G/l and 1.21 ± 0.07 G/l, $p > 0.05$). After 2-3 months of treatment, IgA tended to decrease: in patients of the 1st group from 2.28 ± 0.13 G/l during hospitalization to 2.20 ± 0.13 G/l ($p > 0.05$), in the 2nd group – from 2.01 ± 0.23 G/l to 1.60 ± 0.23 G/l ($p > 0.05$), and in the 3rd group – from 2.39 ± 0.13 G/l to 2.20 ± 0.09 G/l ($p > 0.05$).

A similar trend was typical for IgM, the amount of which compared to baseline varied in patients of Group 1 – from 1.25 ± 0.15 G/l to 1.15 ± 0.17 G/l ($p > 0.05$), in Group 2 from 1.04 ± 0.17 G/l to 0.96 ± 0.22 G/l ($p > 0.05$) and in Group 3 – from 1.16 ± 0.07 G/l to 1.17 ± 0.08 G/l ($p > 0.05$).

The amount of IgG in patients of the 2nd group was dependent on hormone therapy and was characterized by a decrease from 11.99 ± 0.42 G / l to 10.35 ± 0.25 G/l ($p < 0.05$), whereas in patients of the 1st and 3rd groups this indicator did not change significantly from 12.98 ± 0.31 G/l to 12.17 ± 0.39 G/l ($p > 0.05$) and from 12.80 ± 0.34 G/l to 12.03 ± 0.15 G / l ($p > 0.05$), respectively.

The effect of corticosteroid therapy regimens on the phagocytic system after 2-3 months of treatment was ambivalent. Thus, in patients of the 1st group, the functional activity indicators PMNs and MPS in comparison with the initial level, respectively, showed a tendency to increase the neutrophil phagocytic rate (NPR), neutrophil phagocytic count (NPC), monocytes phagocytic activity (MPA) and monocyte phagocytic count (MPC) from $45.5 \pm 2.3\%$ to $49.8 \pm 2.4\%$ ($p > 0.05$), from 2.4 ± 0.3 to 2.7 ± 0.2 ($p > 0.05$), from $29.5 \pm 2.2\%$ to $33.7 \pm 0.2\%$ ($p > 0.05$) and from 1.5 ± 0.2 to 1.7 ± 0.2 ($p > 0.05$).

In patients of the 2nd group, phagocytosis indices remained at the level of initial values with a downward trend and, respectively, amounted to $53.2 \pm 1.5\%$ and $51.5 \pm 1.5\%$ ($p > 0.05$), 2.5 ± 0.1 and 2.4 ± 0.1 ($p > 0.05$), $28.6 \pm 0.9\%$ and $28.4 \pm 1.0\%$ ($p > 0.05$), 1.8 ± 0.1 and 1.7 ± 0.3 ($p > 0.05$).

And only in patients of the 3rd group there an increase of these indicators: from $50.8 \pm 1.6\%$ to $57.1 \pm 1.4\%$ ($p < 0.05$), from 2.5 ± 0.1 to 2.9 ± 0.1 ($p < 0.01$), from $32.1 \pm 1.4\%$ to $33.8 \pm 1.3\%$ ($p > 0.05$) and from 1.7 ± 0.1 to 1.8 ± 0.1 ($p > 0.05$).

More accurate results of the corticosteroids effect on phagocyte function were obtained in vitro studies under the influence of a stress dose ($100 \mu\text{g}\%$) of cortisol. The NPR, NPC, MPA and MPC indices in healthy people decreased, respectively, from $72.8 \pm 0.98\%$ to $50.5 \pm 2.2\%$ ($p < 0.01$), from 3.96 ± 0.12 to 2.80 ± 0.14 ($p < 0.01$), from $44.0 \pm 1.0\%$ to $35.8 \pm 1.34\%$ ($p < 0.01$), and from 3.3 ± 0.14 to 2.5 ± 0.17 ($p < 0.05$).

Similar changes in these indicators of phagocytic function of PMNs and MPS were found in patients with tuberculosis. The decrease in NPR, NPC, MPA and MPC was from $54.3 \pm 1.6\%$ to $38.9 \pm 1.9\%$ ($p < 0.01$), from 2.8 ± 0.14 to 2.23 ± 0.09 ($p < 0.01$), from $37.2 \pm 1.1\%$ to $31.3 \pm 1.4\%$ ($p < 0.01$), and from 2.1 ± 0.08 to 2.0 ± 0.1 ($p < 0.05$).

The study showed that antibacterial therapy received by patients with tuberculosis is directed against the pathogen and, according to the results of Group 1, had no direct effect on immunological protection. The use of corticosteroids in the complex treatment of patients with tuberculosis had an effect

on various parts of the body's immune defense and depended on the administration regimen. With daily administration, GCs suppressed T-cell and phagocytic immunity, as evidenced by their performance after 2-3 months of treatment of patients of Group 2, in which they were reduced and corresponded to the level of the initial values detected during hospitalization, which explained the reasons for the development of complications therapy with GCs. At the same time, when GCs were administered for a long time, they inhibited the functional activity of mononuclear phagocytes (MNF) to a lower level of migration and adhesion, and inhibited chemotaxis and functional activity [9].

GCs also affected PMNs, reducing the superoxide production, inhibiting the release of lysosomal enzymes, and with prolonged action it suppressed the cytotoxic effect of phagocytes, which led to a decrease in phagocytic protection [1].

In patients with tuberculosis, changes in the functional activity of peripheral blood phagocytes showed only a downward trend, while in vitro addition of hydrocortisone to the incubation medium in a stress dose showed a statistically significant suppressive effect on PMNs and MPS in both healthy volunteers and patients with tuberculosis.

The results of examination of patients of the 2nd group after 2-3 months of chemotherapy showed that GCs in the daily administration regimen caused a decrease in peripheral blood of the total number of lymphocytes and their T-helper (CD4+) subpopulation. This could be due to the redistribution of lymphocytes in the organism. However, it is known that GCs can inhibit the ability of T-helper cells (CD4+) to proliferate, and moreover, they can be an inducer of apoptosis of various subpopulations of lymphocytes [4]. Hypersecretion of cortisol is a natural factor in the induction of programmed death of peripheral blood leukocytes, which makes it a logical and understandable immunosuppressive effect of GCs in patients of Group 2 when administered daily.

With the administration of GCs in the morning, every other day, there was a positive effect on T-cell immunity and phagocytosis. Indices of T-cell and phagocytic immunity in patients of the 3rd group reached normal levels after 2-3 months of hormone therapy, which can be explained by the preservation (restoration) of the synchrony of GCs administration and physiological biorhythm of HPA axis activity. The results showed that pharmacotherapeutic doses of corticosteroids when administered taking into account the circadian rhythm of HPA axis function played the role of a physiological regulator of the immune system and had an immunostimulatory effect on lymphocytes. The absence of immunosuppressive effects of low doses of corticosteroids has been pointed out by other authors [7].

The lack of influence of GCs on humoral immunity in our studies confirmed the fact that mature B-lymphocytes are not sensitive to their action [8].

Therefore, the GCs therapy by synchronization with the HPA axis functional activity showed that under such conditions, corticosteroids have a positive effect on T-cell and phagocytic immunity, as evidenced by a significant increase in the total number of peripheral blood lymphocytes, as well as subpopulations of T-lymphocytes after 2-3 months of treatment of patients receiving corticosteroids in the intermittent regimen, which contributed to the normalization of the physiological biorhythm of the HPA axis functional activity, restored the regulatory effect of the neuroendocrine system on the immune defense system, showing an immunostimulatory effect.

Conclusions

1. Antibacterial drugs used in the treatment of tuberculosis have virtually no effect on the immune system at the end of the intensive phase of treatment.
2. Supplementation of antibacterial therapy with corticosteroids in the daily administration regimen with dose tapering after completion of the intensive phase of treatment of tuberculosis has a negative impact on the indicators of immunological protection: GCs suppress the restoration of T-cell and phagocytic immunity and reduce IgG levels without affecting other indicators of humoral immunity.
3. GCs drugs when administered taking into account the physiological biorhythm of the HPA axis functional activity, in the morning, once a daily dose, every other day give the opportunity to perform hormone therapy in a pharmacotherapeutic dose throughout the course of the intensive phase of complex treatment of tuberculosis with drug withdrawal without dose tapering. GCS have a stimulating effect on T-cell and phagocytic immunity, which is manifested by the normalization of their indices by the end of the intensive phase of treatment.

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Реферати

**ГЛЮКОКОРТИКОЇДИ
ЯК ІМУНОСТИМУЛЯТОРИ
В ПАТОГЕНЕТИЧНІЙ ТЕРАПІЇ ТУБЕРКУЛЬОЗУ**

Ярешко А. Г., Куліш М. В.

В статті обґрунтований імуностимулюючий ефект кортикостероїдів в комплексному лікуванні хворих на туберкульозу при введенні їх в подвійній фізіологічній дозі, через день з урахуванням добового біоритму функції гіпоталамо-гіпофізарно-надниркової системи, тоді як при щоденному введенні вони проявляють імуносупресивний ефект. В-лімфоцити периферійної крові не чутливі до дії глюкокортикостероїдних препаратів.

Ключові слова: туберкульоз, патогенетична терапія, глюкокортикостероїди.

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**ГЛЮКОКОРТИКОИДЫ КАК
ИММУНОСТИМУЛЯТОРЫ
В ПАТОГЕНЕТИЧЕСКОЙ ТЕРАПИИ ТУБЕРКУЛЕЗА**

Ярешко А. Г., Кулиш М.В.

В статье обоснован иммуностимулирующий эффект кортикостероидов в комплексном лечении больных туберкулезом при введении их в двойной физиологической дозе, через день с учетом суточного биоритма функции гипоталамо-гипофизарно-надпочечниковой системы, тогда как при ежедневном введении они проявляют иммуносупрессивный эффект. В-лимфоциты периферической крови не чувствительны к действию глюкокортикостероидных препаратов.

Ключевые слова: туберкулез, патогенетическая терапия, глюкокортикостероиды.

Рецензент Костенко В.О.

P.P. Antonenko, V.V. Zazharskyi, N.I. Suslova, P. M. Sklyarov, O.P. Reshetchenko¹,V.K. Kostyuk², R.V. MylostyyviDnipro State Agrarian and Economic University, Dnipro, ¹ Odesa State Agrarian University² National University of Life and Environmental Sciences of Ukraine, KyivEFFICACY OF HERBAL ESSENTIAL OILS AT TETRAHLORMETHANE INDUCED
HEPATITIS IN LABORATORY RATS

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In this paper, the efficacy of essential oils in the composition of Fitohol herbal medicinal product against the background of tetrachloromethane induced toxic hepatitis in laboratory rats has been studied. It was established that the injection of Fitohol in rats at the dose of 0.03 g per 100 g of body weight positively influenced the clinical condition of animals, facilitated the manifestation of hepatocellular failure and the development of destructive changes in hepatocytes (reducing the activity of alkaline phosphatase, alanine and aspartate aminotransferases in blood plasma and oxyproline, hexosamines and malonic dialdehyde in liver homogenates) in acute toxic hepatitis. In the long-term intoxication of rats with CCl₄, the effect of Fitohol was manifested in improving the functional state of the hepatobiliary system and some restoration of the protein- synthesizing liver function.

Key words: herbal medicinal product, tetrachloromethane induced toxic hepatitis, blood plasma, liver homogenate, white rats.

The study is a fragment of the research project "Experimental and theoretical substantiation of methods for diagnostics, treatment and prevention of polymetabolic and polyorganic internal pathology, use of biologically active substances in animals of different species", state registration No. 0118U004022.

The liver is one of the most important organs playing a decisive role in the physiological processes of the body. Along with its important functions in the metabolism (accumulation of glycogen, decomposition of erythrocytes, production of hormones and blood plasma proteins), it plays a central role in the detoxification and transformation of chemicals, and therefore to some extent it is exposed to their harmful effects, which increases its susceptibility to diseases [10].

Treatment and prevention of liver diseases at the early stages, when pathological changes in its structures have not become irreversible, is important. However, synthetic pharmacological drugs used to treat liver diseases are often toxic and have many side effects [9]. Therefore, considerable attention is paid to the possibility of using alternative medicinal preparations, which are natural products or their derivatives. Among them, plant-based preparations, the main advantage of which is safety and long-term therapeutic potential, rank high.

Due to the presence of biologically active substances (essential oils, flavonoids, glycosides, tannins, organic acids, etc.), they have hepatoprotective, choleric and detoxification properties [14], antibacterial and antioxidant activity [8, 15], reduce the level of glucose and cholesterol [5], prevent the development of malignant tumors [6]. Thus, the use of phytopreparations for the purpose of treating pathological conditions of the body is a promising trend. However, the ratio and content of the active plant components in them should be the subject of careful study, since some of them in large quantities may have strong toxic properties [3, 6].

Modeling of pathological conditions in laboratory animals has become widespread in experimental humane and veterinary medicine, including the use of tetrachloromethane (CCl₄) for this purpose, administration of which to rats permits to cause various forms of liver pathology in animals - from acute and chronic hepatitis to its cirrhosis of varying manifestation degrees [2, 14]. Toxic hepatitis in animals is accompanied by changes in the intensity and direction of biochemical processes, metabolic disorders and organ structures at the cellular level, and therefore the study of biochemical blood parameters of rats is an informative method for determining the disorders of the synthetic and excretory function of the liver against the background of using medicinal products.

The purpose of the work was to study the hepatoprotective effect of essential oils in the composition of Fitohol herbal medicinal product in nonlinear males of white rats against the background of toxic hepatitis induced by administration of tetrachloromethane (CCl₄).

Materials and methods. The model of the toxic liver damage caused by an organic hepatotropic poison - carbon tetrachloride (tetrachloromethane, CCl₄) was taken as a basis [2]. A solution of

tetrachloromethane was prepared of the pure (99.99% purity) preparation by adding olive oil. The final concentration of the solution was 50%. Experiments were carried out on sexually mature nonlinear males of white rats aged 6 months with a weight of 0.17-0.18 kg, which were selected on the basis of analogues [1]. They were kept on a standard vivarium diet of the Dnipro State Agrarian and Economic University. All animals had free access to food and drinking water.

The registered Fitohol herbal medicinal product (TU U 10.9-32490422-004: 2012) is a mixture of herbal raw material and medicinal products extracts (tinctures), which, as a medicinal product, contains: *Capsella bursa-pastoris* herb extract (*Capsella bursa-pastoris* (L.) Medik.), *Helichrysum arenanum* flowers extract (*Helichrysum arenarium* L.), *Tanacetum vulgare* flowers extract (*Tanacetum vulgare* L.), peppermint tincture (*Mentha piperita* L.), common valerian root tincture (*Valeriana officinalis* L.), common belladonna leaves tincture (*Atropa belladonna* L.), as well as magnesium sulfate, sodium salicylate, hexamethylenetetramine, glycerol, ethanol.

The experiments were carried out in two stages. At the first stage the efficacy of Fitohol herbal preparation was studied for treating acute tetrachloromethane-induced toxic hepatitis. Laboratory animals were divided into groups: the control group rats (n = 36) were injected subcutaneously into the shoulder with 50% CCl₄ oil solution in the dose of 0.5 ml per 100 g of body weight once a day three times a week, and the experimental group animals (n = 36) were given Fitohol from the first day on the background of CCl₄ in the dose of 0.03 g per 100 g of body weight daily for 30 days. Euthanasia of the experimental animals was performed on the 7th, 14th and 30th day of the experiment.

At the second stage, biochemical changes in blood plasma against the background of long-term intoxication of rats with CCl₄ were determined. Animals of the control group (n = 15) were subcutaneously injected 50% CCl₄ oily solution in the dose of 0.05 ml per 100 g of body weight 2 times a week; rats of experimental group II (n = 15), against a background of toxic hepatitis, Fitohol was given in the dose of 0.03 g per 100 g of body weight daily from the 4th week for the period of 16 weeks. Biochemical parameters of laboratory animals' blood plasma were studied after their euthanasia the day after the last administration of the herbal preparation. Separately, at each stage of the study, groups of intact animals were formed (n = 12 and n = 15).

Clinical signs of toxic hepatitis in experimental rats were estimated by a set of indices: appearance, behavior, appetite, body weight, skin elasticity, the coat state [1]. During the experiment, the requirements of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Scientific Purposes" (Strasbourg, 1986), the Law of Ukraine "On the Protection of Animals from Cruel Treatment" No. 3447 dated February 21, 2006, were observed. The material for the study was plasma and liver homogenate.

Euthanasia of animals was performed with sodium thiopental. To obtain plasma, blood was taken from the heart of rats into heparin containing test tubes, which were then centrifuged at 3000 rpm for 15 minutes. Homogenate for biochemical studies was obtained from the animal samples of the normal and pathologically altered left side lobe of the liver (25 g) after pre-perfusion with physiological solution by means of differential homogenization.

Laboratory study was carried out at the clinical biochemistry laboratory of the Research Centre of Biosafety and Environmental Control of Agro-Industrial Complex (Dnipro). The plasma levels of cholesterol, bile acids, β -lipoproteins, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (AP) activity; in the liver homogenates, the total proteins content, oxyproline (OP), hexosamines (HA) and malonic dialdehyde (MDA) were determined with "Miura" automated biochemical analyzer (Italy) in compliance with the recommendations of the Panel of Experts of the International Federation of Clinical Chemistry (IFCC) using the High Technology reagent kits (USA), PZ Cormay S.A. (Poland) and Spinreact S.A. (Spain).

The results were processed using the Statistica 10 software package (StatSoft Inc., USA). Reliability of the difference between the samples was assessed according to the Student's t-criterion, having first checked the normality of their distribution. Differences were considered reliable for $P < 0.05$.

Results of the study and their discussion. Observations have established that in animals' intoxication with tetrachloromethane, Fitohol mitigated the course of the pathological process. On the second day after CCl₄ administration to rats (control group), lethargy, adynamia, and loss of appetite were observed. Signs of intoxication grew and were manifested to the fullest extent on the 5th day, and then gradually faded. In the rats, which were administered Fitohol simultaneously with CCl₄ (experimental group I), the phenomena of intoxication came later (on the 4th-5th days) and were less pronounced. The external signs of poisoning within two weeks have completely disappeared, while in control animals they were still manifested.

Mechanisms of haloalkanes hepatotoxic action, including CCl₄, are associated with the development of centrilobular necrosis, infiltration of the liver by inflammatory cells, fatty dystrophy and apoptosis of hepatocytes. The AP activity increase in the control group rats compared to the experimental ones by 1.7-3.9 times ($p = 0.031-0.0004$) may indicate a worsening of bile outflow, which, as a rule, tends to complicate hepatitis (table 1).

Table 1

Activity of rats' blood serum enzymes in acute toxic hepatitis ($M \pm m$, $n = 12$)

Group	Toxic hepatitis, days	Enzymes activity		
		ALT, U/l	AST, U/l	AP, U/l
control (CCl ₄)	7	138.3±7.19	78.3±7.20	947.8±5.40
Experimental I (CCl ₄ +Fitohol)		128.4±3.96	72.2±6.99	342.7±3.59*
control (CCl ₄)	14	50.4±3.23	66.1±5.04	708.8±11.27
Experimental I (CCl ₄ + Fitohol)		48.0±3.51	57.6±3.59	180.3±6.11*
control (CCl ₄)	30	90.1±5.32	102.7±6.11	702.7±6.69
Experimental I (CCl ₄ + Fitohol)		60.2±3.60*	90.5±5.14	420.0±4.82*
Intact	-	54.0±4.10	81.6±4.89	416.4±4.99

Note: * – difference compared to the control group rats at $p < 0.05$.

Significant increase of ALT activity in experimental animals compared to the intact ones (by 2.4-2.6 times) against the background of tetrachloromethane may indicate a development of hepatocellular failure, cytolytic syndrome and biliary obstruction during the first week of toxic hepatitis modeling. At the same time, there was no significant difference between the activity of transaminases (ALT and AST) in the experimental animals, with the exception of ALT in the rats of experimental group I it was lower by 33% at the 30th day of the experiment ($p = 0.0012$) than in the control, which may indicate some cumulative effect of herbal preparations as hepatoprotections.

Development of hypocholesterolemia suggests the presence of destructive changes in the liver parenchyma. In toxic hepatitis (table 2), in the control group animals, during 7 days, serum cholesterol level reduced by 38% ($p = 0.047$) and by 14% by 37% ($p = 0.008$), compared to the first experimental group of rats, which was apparently a result of deep damage to hepatocytes and their synthetic activity disorder. At the same time, the difference between the animals of the first experimental group and the intact rats was unreliable. There were also no significant differences in the content of bile acids.

Table 2

Dynamics of lipid metabolism indices in blood plasma of rats in acute toxic hepatitis ($M \pm m$, $n = 12$)

Group	Toxic hepatitis, days	Cholesterol, mmol/l	Bile acids, mmol/l	β -lipoproteins, units
control (CCl ₄)	7	0.92±0.07	0.209±0.017	25.9±3.19
Experimental I (CCl ₄ +Fitohol)		1.47±0.11*	0.229±0.019	27.1±1.34
control (CCl ₄)	14	0.82±0.03	0.186±0.007	191.7±11.23
Experimental I (CCl ₄ + Fitohol)		1.29±0.08*	0.207±0.026	124.2±15.73*
control (CCl ₄)	30	1.52±0.14	0.379±0.029	108.9±9.74
Experimental I (CCl ₄ + Fitohol)		1.46±0.29	0.331±0.034	80.6±6.86*
Intact	-	1.80±0.12	0.188±0.035	11.1±1.38

Note: * – difference compared to the control group rats at $p < 0.05$.

The normal level of lipoproteins provides the most important stage in the elimination of lipids from the blood. Cholesterol, which is transported in their composition serves as a source for the synthesis of bile acids and, thus, leaves the body. It was found that the blood serum β -lipoprotein concentration in experimental group I reduced by 35% on the 14th day ($p = 0.0073$) and it was lower than control by 26% on the 30th day ($p = 0.049$). This may indicate an improvement in the course of animals' lipid metabolism in response to Fitohol.

Increase of the HA content indicates an increase in the decomposition of carbohydrate-protein components of the connective tissue and the inflammatory process activity, the duration of which leads to the destruction of liver tissues. In our case (table 3), their growth in rat homogenate of the liver tissues 7 days after the initiation of CCl₄, may indicate the development of the necrobiotic process in hepatocytes and the presence of inflammatory process. It was more pronounced in the control rats, since the level of HA in them was higher on the 7th day - by 22% ($p = 0.046$) and on the 30th day - by 9% ($p = 0.0009$) than in the experimental group.

Oxyproline (OP) is one of the main amino acids of collagen, which permits to consider it as a marker, which reflects catabolism of this protein during the liver fibrosis development. It was found that on the 7th day after artificially induced liver inflammation there was an increase in the content of OP,

indicating the development of destructive changes in the liver tissues. In the control group, it was higher by 54% ($p = 0.0004$) than in the experimental group rats, the content of oxyproline in which, due to the action of Fitohol, remained within the limits of intact animals. On the 14th day, its concentration increased in rats of all groups, but was lower by 18% ($p = 0.021$) than in the control. The tendency to reduce the content of oxyproline in liver tissue homogenates up to the 30th day of observation occurred in both groups, and only in the experimental group it was at the level of intact animals.

Table 3

Dynamics of rat liver homogenates biochemical indices in acute toxic hepatitis ($M \pm m$, $n = 12$)

Group	days	Proteins, mg/1g tissue	Oxyproline, $\mu\text{mol}/\text{mg}$ protein	Hexosamines, $\mu\text{mol}/\text{mg}$ protein	MDA, $\mu\text{mol}/\text{mg}$ protein
control (CCl ₄)	7	63.8 \pm 7.48	33.9 \pm 2.19	210.4 \pm 16.18	0.414 \pm 0.021
Experimental I (CCl ₄ +Fitohol)		99.3 \pm 5.97*	15.7 \pm 1.17*	163.5 \pm 12.69*	0.154 \pm 0.018*
control (CCl ₄)	14	54.0 \pm 4.6	26.5 \pm 1.39	168.1 \pm 13.08	0.218 \pm 0.058
Experimental I (CCl ₄ + Fitohol)		63.4 \pm 1.87	21.8 \pm 1.52*	163.9 \pm 15.69	0.259 \pm 0.006
control (CCl ₄)	30	68.6 \pm 4.01	19.8 \pm 2.63	469.1 \pm 5.37	0.139 \pm 0.013
Experimental I (CCl ₄ + Fitohol)		75.0 \pm 9.1	11.8 \pm 2.46	425.4 \pm 2.95*	0.151 \pm 0.024
Intact	-	73.9 \pm 3.73	15.4 \pm 4.09	88.8 \pm 6.03	0.208 \pm 0.042

Note: see table 1.

Assumption about the protective properties of Fitohol in the initial period (7th day) of acute toxic hepatitis is confirmed by the level of malondialdehyde (MDA) in the rat liver tissue homogenates. Due to its significant prevalence in the control group animals (by 2.7 times; $p = 0.0031$), the MDA content in the experimental rats was even lower than the level of the intact animals. On the 14th and 30th day there was a tendency towards normalization of this index in the control group, and therefore the content of MDA in rats of all groups did not differ significantly. Prevention of the malondialdehyde accumulation - the final product of lipid peroxidation in liver tissue homogenates - due to the effect of Fitohol suggests that this herbal medicinal product also has a protective and antioxidant effect on cellular membranes of hepatocytes.

In the long-term administration of CCl₄ (for 16 weeks) to white rats (control) in their blood serum (table 4), compared to the intact animals, there was a decrease in the total protein content - by 1.2 times, cholesterol - by 1.7 times, a significant increase in the content of bilirubin - by 2.7 times, in cholesterol - by 1.5 times, in the activity of ALT - by 3.5 times, and in alkaline phosphatase - by 4.9 times, which may indicate a significant damage to hepatocytes and disorders of metabolic processes against the background of cholestasis development.

Table 4

Biochemical indices of rat blood plasma in CCl₄-induced toxic hepatitis within 16 weeks ($M \pm m$, $n = 15$)

Index	Measuring units	Group		
		Control	Experimental II	Intact
Total protein	g/l	59.6 \pm 3.33	66.5 \pm 4.41	72.8 \pm 3.12
Bilirubin	$\mu\text{mol}/\text{l}$	32.4 \pm 6.92	10.4 \pm 2.51*	12.0 \pm 3.43
Cholesterol	mmol/l	1.02 \pm 0.14	1.56 \pm 0.11*	1.74 \pm 0.15
ALT	U/l	265.2 \pm 2.75	118.2 \pm 4.23*	75.6 \pm 4.53
AP	U/l	1843.7 \pm 6.43	940.3 \pm 8.19*	375.5 \pm 5.85

Note: * - difference compared to the control group rats at $p < 0.05$.

Administration of Fitohol from the 4th week after CCl₄ administration (experimental group II) had a positive effect on the liver cells function, reducing the content of bilirubin and ALT activity in rats, respectively, by 3.1 ($p = 0.047$) and by 2.2 ($p = 0.0002$) times compared to the control group. The AP activity was reduced almost twice ($p = 0.0004$), but signs of cholestasis were observed in the future. The trend towards a higher total protein content by 11.6% and cholesterol by 53% ($p = 0.0331$) in rats under Fitohol effects may indicate some recovery of the liver protein-synthesizing function and lipid metabolism improvement in the long-term intoxication with tetrachloromethane.

Metabolic processes are primarily controlled by the liver and its disruption contributes to liver injury. Since the changes associated with CCl₄ induced liver damage are similar to that of acute viral hepatitis [4], the ability of a hepatoprotective drug to lessen the harmful effects or to preserve the normal hepatic physiological mechanisms, which have been distressed by a hepatotoxin, is the index of its protective effects. It is known that with toxic hepatitis [12], the increase in membrane permeability causes leakage of liver enzymes (ALT, AST and ALP) into blood circulation as shown by abnormally high levels of serum hepatic markers. The results of present study is in agreement with studies the authors' [7, 14] in which extracts of medicinal plants causes concomitant reduction in the serum enzyme activities of ALT, AST and ALP as

compared to the toxicant ethanol and carbon tetrachloride treated group, whereas in present study such a reduction in serum enzyme levels is due to herbal medicinal product (Fitohol) is also observed. As well as other researchers [13], we tend to believe that the protective effects of essential oils in the composition medicinal plants in when artificially induced toxic hepatitis in laboratory rats can be explainable attributed to the cell membrane stabilizing ability of the extracts, preventing the liver dysfunction and the transfer of enzymes into the serum. The hepatoprotective effect of plant extracts was also associated [4, 14] with the presence of antioxidant compounds in them, which increased the level of antioxidant enzymes and prevented the accumulation of lipid peroxidation products. A similar effect was confirmed in our study, when the level of MDA in acute toxic hepatitis was several times lower with the use of Fitohol. In summary, we assume that the high hepatoprotective efficacy of Fitohol may be due to the synergistic and supra-additive action of essential oils in medicinal plants included in its composition, also as mentioned by other researchers [11].

Conclusions

1. It was established that under the conditions of simulated CCl₄-induced toxic hepatitis in white rats there was a deterioration of the general clinical condition, development of hepatocellular failure, cholestasis and degenerative changes in the liver, which is confirmed by the biochemical parameters of blood plasma and its homogenates.
2. It was found that application of Fitohol to animals with toxic hepatitis contributed to normalization of a number of biochemical indices reflecting the functional state of the hepatobiliary system, opening the possibility of using this herbal medicinal product as a hepatoprotective agent.

Prospects for further research lie in determining the dose and developing schemes for the use of Fitohol in the treatment of small domestic and productive animals in the gastrointestinal tract pathology and other concomitant diseases.

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Реферати

ЭФЕКТИВНІСТЬ РОСЛИННИХ ЕФІРНИХ ОЛІЙ ЗА ТЕТРАХЛОРМЕТАНОВОГО ГЕПАТИТУ В ЛАБОРАТОРНИХ ЦУРІВ

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У роботі вивчена ефективність застосування ефірних олій у складі рослинного препарату фітохол на тлі тетрахлорметанового токсичного гепатиту в

ЭФФЕКТИВНОСТЬ РАСТИТЕЛЬНЫХ ЭФИРНЫХ МАСЕЛ ПРИ ТЕТРАХЛОРМЕТАНОВОМ ГЕПАТИТЕ У ЛАБОРАТОРНЫХ КРЫС

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В работе изучена эффективность применения эфирных масел в составе растительного препарата фитохол на фоне тетрахлорметанового токсического гепатита у лабораторных

лабораторних щурів. Встановлено, що випоювання фітохолу щурам в дозі 0,03 г на 100 г маси тіла позитивно впливало на клінічний стан тварин, полегшувало прояв гепатоцелюлярної недостатності та розвиток деструктивних змін в гепатоцитах (знижуючи активність лужної фосфатази, аланін- і аспартатамінотрансферази в плазмі крові та оксипроліну, гексозамінів та малонового діальдегіду – в гомогенатах печінки) за гострого токсичного гепатиту. За тривалої інтоксикації організму щурів CCl_4 дія фітохолу проявлялася в покращенні функціонального стану гепатобіліарної системи та деяким відновленням білоксинтезуючої функції печінки.

Ключові слова: рослинний препарат, тетрахлорметановий токсичний гепатит, плазма крові, гомогенат печінки, білі щури

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крыс. Установлено, что выпойка фитохола крысам в дозе 0,03 г на 100 г массы тела положительно влияла на клиническое состояние животных, облегчала проявление гепатоцеллюлярной недостаточности и развития деструктивных изменений в гепатоцитах (снижая активность щелочной фосфатазы, аланин- и аспартатаминотрансферазы в плазме крови и оксипролина, гексозаминов и малонового диальдегида – в гомогенатах печени) при остром токсическом гепатите. При длительной интоксикации организма крыс CCl_4 действие фитохола проявлялось в улучшении функционального состояния гепатобилиарной системы и некоторым восстановлением белоксинтезирующей функции печени.

Ключевые слова: растительный препарат, тетрахлорметановый токсический гепатит, плазма крови, гомогенат печени, белые крысы.

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PROTECTIVE EFFECT OF QUERCETIN ON THE ORAL CAVITY TISSUES IN RATS IN THE PRESENCE OF GENOTOXICANT AND ALIMENTARY DEFICIENCY OF POLYPHENOLS

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The purpose of the study was to establish the effect of the flavonoid quercetin on the oral cavity tissues in rats in the presence of genotoxicant fluorouracil with insufficient intake of plant polyphenols. The experiment was carried out on 21 white rats of the Wistar line. Intact animals were kept on a standard vivarium diet. The control group received a 5% fluorouracil solution *per os* against the background of a flavonoid diet; freshly prepared 7.5% potato starch gel was applied to the oral mucosa of the rats. Group 3 rats were additionally treated daily with a gel containing quercetin at a dose of 0.25 mg/kg. The experiment lasted 70 days. The gums turned out to be more sensitive than the buccal mucosa to long-term exposure to the genotoxicant fluorouracil in the case of nutritional deficiency of plant polyphenols. Quercetin in the accepted experimental conditions showed a significant anti-inflammatory effect in the buccal mucosa.

Key words: fluorouracil, nutritional deficiency of plant polyphenols, quercetin, gums, buccal mucosa, protective effect, rats.

The study is a fragment of the research project "The effect of hypoxia on the processes of collagen formation and mineralization in models of dental pathology and correction of these disorders", state registration No. 0118U006963.

The occurrence of dental morbidity has recently increased due to the increased content of toxicants in the external environment. The consumption of medicinal xenobiotics has also increased significantly.

Fluorouracil is an antitumor agent used in mono- or polychemotherapy, is a structural analogue of pyrimidine, in terms of chemical structure – 2,4-dioxy-5-fluoropyrimidine. It is known that pyrimidine bases: cytosine, uracil, thymine are a part of nucleic acids. In the organism, fluorouracil can enter into a competitive relationship with uracil, being its antimetabolite, and thus turns out to be a biochemical substrate for enzymes responsible for the metabolism of uracil. Along with inhibition of DNA bases synthesis, the drug can be included in both DNA and RNA, and thus lead to inhibition of DNA synthesis and all RNA fractions, because the functioning of DNA as a DNA matrix is disrupted [12]. Considering all of the above, fluorouracil is a genotoxicant. It is classified as a highly toxic substance. When using fluorouracil, there may be pronounced changes in peripheral blood, inhibition of bone marrow hematopoiesis, hemorrhagic phenomena of various localization, stomatitis, ulceration of the mucous membrane of the oral cavity and digestive tract.

Recent studies have shown the diverse effects of plant polyphenols (including flavonoids) on a living organism: antioxidant, anti-inflammatory, cytoprotective, antimicrobial, antiviral, etc.

Currently, the antioxidant activity of flavonoids is paid great attention as a possible mechanism through which the biological effects of this group of compounds are realized [10]. For a long time, flavonoids have been considered as the most important antioxidants [15]. Their defining chemical property

is the tendency to easily give up protons. When oxidized, due to the conjugation of redox reactions, they promote the reduction of other biologically active substances or prevent their oxidation [11]. The antioxidant activity of flavonoids allows to normalize the processes of free radical oxidation of organic molecules and the level of peroxide radicals accumulating in the cells of the organism, which is important for the construction and renewal of structural lipids of cell membranes, for the production of a number of hormones.

The main functional groups that determine the chemical activity, biochemical and pharmacological action are phenolic hydroxyls. The more hydroxyl groups a flavonoid has, the stronger it is as an antioxidant [13]. In nature, the most common are flavonoids with 4 or 5, less often 1,2 or 6-OH groups in the molecule. The strongest antioxidant effect was found in quercetin (3,5,7,3', 4'-OH and the presence of $C_2 = C_3$), thus it contains 5 OH groups and has a wide spectrum of biological activity.

Particular attention is drawn to its antioxidant, antiradical, anti-inflammatory properties. The ability of quercetin to give up a hydrogen atom to one of the hydroxy groups and form a free phenoxy radical stabilized by an unpaired electron leads to the termination of the free radical oxidative chain reaction. Quercetin is localized near the surface of the lipid bilayer of membranes, and therefore has a stronger antioxidant effect [14].

Recently, a significant number of facts have accumulated indicating the essential role of alimentary plant components in maintaining the resistance of the oral cavity tissues to the damaging effect of factors of various nature. Despite the diverse properties of quercetin, its application in dentistry is still very limited.

The purpose of the study was to establish the effect of quercetin on the oral cavity tissues of rats under the action of genotoxicant fluorouracil under the conditions of nutritional deficiency of plant polyphenols.

Materials and methods. The study was carried out on 21 white Wistar breeding rats. Intact animals (7 animal units) were kept on a standard vivarium diet. In the second, control group, 7 rats on the background of a polyphenolic diet received *per os* 5% fluorouracil solution (pyrimidine uracil antimetabolite, manufactured by the pharmaceutical company PC "Darnitsa", Ukraine) at a dose of 12.5 mg/kg of body weight of rats. Diet without flavonoids [5] contained: wheat flour – 30%, whole milk powder – 30%, starch – 20%, sugar – 15%, sodium chloride – 1%. Alfalfa flour, which contains plant flavonoids, was excluded from the diet. Application of the gel containing freshly prepared 7.5% potato starch was carried out using a dispenser on the mucous membrane of the rats' oral cavity. Rats of the 3rd group (7 animals) were kept on a low-polyphenol diet and received *per os* a solution of fluorouracil, as well as a gel to control the effect of the carrier of the studied drug. As part of the gel, the rats of the 3rd group were daily applied quercetin (granules produced by PJSC SIC "Borshchahivskiy CPP", Ukraine) at a dose of 0.25 mg/kg. The experiment lasted 70 days.

At the end of the experiment, the rats were sacrificed by total exsanguination from the vessels of the heart under anesthesia with thiopental (40 mg/kg). Having previously separated the gums and buccal mucosa, the upper and lower jaws were dissected out and subjected to morphometric examination [7]. The objects of biochemical studies were blood serum, supernatant of rat liver, gum and buccal mucosa homogenates. The supernatant of the homogenates was obtained by centrifugation in an OS-6 centrifuge at 3000 rpm for 15 minutes.

The lipid peroxidation level (LPL) process was assessed by the content of malondialdehyde (MDA). The state of the physiological antioxidant system was assessed by the activity of antioxidant enzymes: catalase [1], superoxide dismutase (SOD) [9], glutathione peroxidase [2]. The activity of acid phosphatase was assessed by the method [6], elastase – by the method [4].

Experimental results were processed by statistical methods with the determination of t-criteria for the reliability of differences according to Student's t-test.

Results of the study and their discussion. Daily oral administration of the toxicant fluorouracil to rats was carried out by keeping them on a low-polyphenol diet, which the animals tolerated well. Weight gain was the same as in the intact group. There were no differences in the behavior of the rats and their appearance.

Consider how the studied biochemical parameters in the blood serum, liver and oral mucosa of rats changed after 70-day experimental exposure.

The use of fluorouracil in low-polyphenolic insufficiency did not significantly affect the MDA content in the studied tissues (table 1). At the same time, with the specified pathogenic effect, the activity of antioxidant enzymes significantly decreased. Thus, the activity of catalase in the liver decreased by 13.4% ($p > 0.05$). More significantly, by 7.1 times ($p < 0.001$), the SOD activity decreased in this research object (table 1).

MDA content and activity of antioxidant enzymes in rat tissues (M±m; p; p₁)

Studied indices	Groups of animals		
	Intact	Control (C)	C+quercetin
	Liver		
Content of MDA (μmol/g)	59.9±4.10	40.3±0.98	51.6±5.60
Activity:			
catalase (mkat/g)	920±4.70	797±18.1	904±5.7 p ₁ <0.001
SOD (RU)	0.64±0.017	0.090±0.024 p<0.001	0.073±0.020 p ₁ <0.001
	gum		
Content of MDA (μmol/g)	89.9±7.02	68.2±18.7	90.0±17.7
Activity: SOD (RU)	0.55±0.090	0.33±0.069 p=0.06	0.20±0.033
Glutathione peroxidase (mkat/g)	222±24.9	93.5±13.9 p=0.006	138±22.2 p ₁ =0.11
	buccal mucosa		
MDA content (μmol/g)	120±14.0	101±15.6	101±17.5
Activity: SOD (RU)	0.30±0.060	0.44±0.084	0.32±0.057
Glutathione peroxidase (mkat/g)	142±11.1	75.0±9.42 p=0.001	88.9±5.45

Note: In tables 1 and 2, the reliability index p was calculated in comparison with the intact group; p₁ – compared with the control.

In the gums, fluorouracil against the background of a low-polyphenolic diet caused a significant inactivation of antioxidant proteins-enzymes. Thus, SOD activity decreased by 60 % (p=0.06); glutathione peroxidase activity – by 42 % (p=0.006; table 2). In the buccal mucosa, the combination of orally administered fluorouracil against the background of a diet without polyphenols reduced the activity of glutathione peroxidase by 1.9 times (p = 0.001) and did not significantly change the SOD activity (table 1).

An increase in inflammatory phenomena in the rats' organisms was evidenced by an increase in the acid phosphatase activity in the blood serum by 1.5 times (p = 0.06) compared with the intact group (Table 2). Another enzyme that accumulates in the focus of inflammation and has a pronounced destructive effect was granulocyte elastase. The activity of blood serum elastase under the action of fluorouracil in a low-polyphenolic diet increased by 4.9 times (p <0.001): 10.0±0.91 nkat/ml versus 2.04±0.76 nkat/ml (table 2).

Table 2

Effect of quercetin on the activity of acid phosphatase and elastase in the blood serum and oral mucosa of rats (M±m; p; p₁)

Groups of animals	Activity	
	acid phosphatase (nkat/ml; nkat/g)	elastase (nkat/ml; nkat/g)
	blood serum	
Intact	78.8±15.7	2.04±0.76
Control (C)	120±12.2 p=0.06	10.0±0.91 p<0.001
C+quercetin	90.5±10.7 p ₁ =0.09	2.07±0.16 p ₁ <0.001
	gum	
Intact	1.30±0.27	1.78±0.13
Control (C)	0.54±0.10	2.81±0.42 p=0.04
C+quercetin	1.15±0.32	2.43±0.57
	buccal mucosa	
Intact	1.27±0.28	2.22±0.28
Control (C)	0.73±0.14	1.83±0.41
C+quercetin	0.28±0.05 p=0.006 p ₁ =0.011	1.93±0.37

The protective effects of quercetin have been studied in the context of oral administration of fluorouracil and reproductive nutritional deficiency of flavonoids. Quercetin has shown periodontal protection properties. It reduced bone resorption of the alveolar bone of rats on the lower jaw by 9% (p₁=0.03): 32.2±1.1% vs. 35.5±0.7%, and by 11 % (p₁=0.03) in the upper jaw: 20.8±0.8 vs. 23.5±0.8% (100% in the control group).

Under the influence of quercetin, administered against the background of fluorouracil and the maintenance of rats on a low-polyphenolic diet for 70 days, the MDA level practically corresponded to that in intact animals in the studied liver tissues and gums (table 1).

Quercetin in the liver normalized the activity of catalase, as well as the activity of SOD in the buccal mucosa of rats in comparison with the data of intact groups (table 1). The activity of glutathione peroxidase under the action of quercetin in the gums and buccal mucosa tended to increase (by 48% and 19%, respectively) compared to the data of the control groups (table 1).

Quercetin under accepted experimental conditions reduced acid phosphatase activity by 75% (trend; $p_1 = 0.09$) in rats' blood serum. At the same time, the activity of elastase decreased by 4.8 times ($p_1 < 0.001$; table 2), which indicates the anti-inflammatory properties of this drug. The activity of acid phosphatase in the gums under the influence of quercetin practically corresponded to the data of the intact group (table 2). At the same time, the activity of elastase in the gums did not change significantly (table 2). In the buccal mucosa, a decrease in acid phosphatase activity was found by 2.6 times ($p_1 = 0.011$) compared with the control group and by 4.5 times ($p = 0.006$) compared with intact (table 2). The activity of elastase under the action of quercetin did not significantly change in the gums and buccal mucosa (table 2).

Studies have shown that the gum, in comparison with the buccal mucosa, turned out to be more sensitive to prolonged experimental exposure, the influence of the genotoxicant fluorouracil in conditions of nutritional deficiency of plant polyphenols. An increase in inflammation was found in the gums. There was also a significant drop in the level of protective proteins-enzymes.

Quercetin, under the accepted experimental conditions, had a significant anti-inflammatory, antioxidant effect in the oral mucosa of rats, which resulted in a decrease in the level of bone resorption processes in the bone tissue of the alveolar process.

We have previously carried out experimental studies on the protective effect of plant polyphenols, which include quercetin, under conditions of reproduction of periodontal pathology in rats using another genotoxicant – dichlorodiphenyldichloroethylene – DDE (the main metabolite of dichlorodiphenyltrichloroethane – DDT) [3]. The preparation of polyphenols (flavonoids and flavone glycosides) is a combination of concentrates of cereal seedlings and the aerial part of yarrow (*Achillea millefolium*) with the code name PF4. The systemic normalizing effect of the drug on transaminase activity, LPL processes, and the activity of antioxidant enzymes – catalase and glutathione exchange enzymes in rats' blood serum was demonstrated [8]. With a topical effect on the oral cavity tissues of rats, the PF4 preparation partially reduced inflammation in the oral mucosa, bone resorption of the alveolar bone in rats, and normalized the mineralization processes in a relatively short period of the experiment (35 days).

The regenerative properties of the PF preparation were studied under conditions of experimental trauma and the administration of genotoxicant DDE [15]. For this, rats under anesthesia (calypsol at a dose of 20 mg/kg, i.p.) underwent a dosed injury to the gingival margin of the incisor. The healing process was monitored for clinical signs of inflammation. The average area of the wound surface in the study group, which received the drug, already on the 2nd day of examination was significantly lower than in other groups. The average area of the wound due to epithelialization under the influence of plant polyphenols on the 7th day of examination decreased by 4.7 times compared with the group “Trauma+DDE”. The final recovery in the group of rats receiving the PF4 preparation occurred on the 10th day. In the group of rats with gum trauma and the administration of the toxicant DDE, it was only on the 13th day. On the 10th day of the experiment, only 50% of recovered animals were found in the group of rats that received the toxicant against the background of trauma [5]. Thus, the genotoxicant DDE aggravated the course of the wound process of the mucous membrane of the rats' gum in case of its dosed traumatic injuries, and the preparation of plant polyphenols under these conditions had a pronounced protective effect on the course of the wound process and the rate of healing of the mucous membrane of the rats' gums.

Experiment demonstrated the osteotropic properties of quercetin and substantiated its use as a therapeutic and prophylactic agent (quercetin granules at a dose of 100 mg/kg of body weight in rats) under conditions of reproduction of experimental periodontitis in rats, caused by their maintenance for three months on a "soft" diet with the maximum reduced load on the periodontium. Under the conditions of modeling periodontitis, the processes of LPL in periodontal tissues were significantly activated, the activity of elastase in the gum increased, and the resorption of the alveolar process bone significantly increased. Oral administration of quercetin significantly reduced the resorptive processes in the periodontal bone tissue, the activity of elastase and acid phosphatase in the gums, which indicated a high therapeutic and prophylactic efficacy of quercetin.

Thus, bioflavonoid quercetin, which has a variety of biological effects, can be recommended as an effective therapeutic and prophylactic agent for dental diseases: gingivitis, periodontitis, etc.

Conclusions

1. Genotoxicant fluorouracil in combination with a diet without plant polyphenols caused an increase in inflammation in the gums of rats and a significant drop in the level of protective enzymes-proteins – SOD and glutathione peroxidase.
2. The flavonoid quercetin has a protective and anti-inflammatory effect. It normalized the activity of the pro-inflammatory enzyme of acid phosphatase in the gums and significantly reduced its activity in the buccal mucosa of rats.
3. Quercetin showed osteotropic action. It reduced the level of resorption processes in the alveolar bone in rats.

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Реферат

**ЗАХИСНИЙ ВПЛИВ ФЛАВОНОЇДУ
КВЕРЦЕТИНУ НА СТАН ТКАНИН
РОТОВОЇ ПОРОЖНИНИ ЩУРІВ
В УМОВАХ ДІЇ ГЕНОТОКСИКАНТА
І АЛІМЕНТАРНОЇ НЕДОСТАТНОСТІ
ПОЛІФЕНОЛІВ**

**Бородач В.А., Шнайдер С.А., Сулова О.В.,
Савельєва Н.Н., Анісімова Л.В., Ткаченко Є.К.**

Метою дослідження було вивчення впливу флавоноїду кверцетину на тканини ротової порожнини щурів при дії генотоксиканта фторурацилу при недостатньому надходженні рослинних поліфенолів. У дослідження було взято 21 білого щура лінії Вістар. Інтактні тварини утримувалися на стандартному раціоні виварію. Контрольна група на тлі бесфлавоноїдного раціону отримувала *per os* 5% розчин фторурацилу, на слизову оболонку порожнини рота щурів наносили гель – свіжоприготований 7,5%-ий картопляний крохмаль. Щурам 3-ої групи додатково щодня наносили гель, до складу якого входив кверцетин в дозі 0,25 мг/кг. Тривалість дослідження склала 70 днів. Десна виявилися більш чутливими, ніж слизова оболонка щоки до тривалого впливу генотоксиканта фторурацилу при аліментарній недостатності рослинних поліфенолів. Кверцетин в прийнятих експериментальних умовах проявив значну протизапальну дію в слизовій оболонці щоки.

Ключові слова: фторурацил, аліментарна недостатність рослинних поліфенолів, кверцетин, ясна, слизова оболонка щоки, захисна дія, щури.

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**ЗАЩИТНОЕ ВЛИЯНИЕ ФЛАВОНОИДА
КВЕРЦЕТИНА НА СОСТОЯНИЕ ТКАНЕЙ
РОТОВОЙ ПОЛОСТИ КРЫС В УСЛОВИЯХ
ДЕЙСТВИЯ ГЕНОТОКСИКАНТА
И АЛИМЕНТАРНОЙ НЕДОСТАТОЧНОСТИ
ПОЛИФЕНОЛОВ**

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Целью исследования явилось изучение влияния флавоноида кверцетина на ткани ротовой полости крыс при действии генотоксиканта фторурацила при недостаточном поступлении растительных полифенолов. В опыт была взята 21 белая крыса линии Вистар. Интактные животные содержались на стандартном рационе вивария. Контрольная группа на фоне бесфлавоноидного рациона получала *per os* 5% раствор фторурацила, на слизистую оболочку полости рта крыс наносили гель – свежеприготовленный 7,5%-ный картофельный крахмал. Крысам 3-ей группы дополнительно ежедневно наносили гель, в состав которого входил кверцетин в дозе 0,25 мг/кг. Длительность опыта составила 70 дней. Десна оказалась более чувствительной, чем слизистая оболочка щеки к длительному воздействию генотоксиканта фторурацила при алиментарной недостаточности растительных полифенолов. Кверцетин в принятых экспериментальных условиях проявил значительное противовоспалительное действие в слизистой оболочке щеки.

Ключевые слова: фторурацил, алиментарная недостаточность растительных полифенолов, кверцетин, десна, слизистая оболочка щеки, защитное действие, крысы.

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PATHOLOGICAL MORPHO-FUNCTIONAL DYSINTEGRATION AS THE KEY PATHOGENETIC MECHANISM OF EXPERIMENTAL LIVER CIRRHOSIS

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The purpose of the study is to investigate the liver cirrhosis pathophysiological mechanisms in rats and to investigate the efficacy of pathogenetical correction of these pathology using L-arginine aspartate and tivortin. Experimental studies proved the lipid peroxidation involvement into the liver cirrhosis pathogenetic mechanisms. The data obtained indicate the cellular apparatus of the blood participation in the pathogenetic mechanisms of the hepatocytes lesions. The data received in morphological studies are comparable with the results of biochemical investigations confirming the unified concept of pathological morpho-functional disintegration development in conditions of investigated pathology that is the basis for the multiple organ failure syndrome development. The authors argue that the positive results of both L-arginine aspartate and tivortin using in experimental liver cirrhosis are supposed to be an experimental background of these pharmacons clinical efficacy testing.

Key words: experimental liver cirrhosis, lipid peroxidation, morphological disturbances, pathophysiological mechanisms, L-arginine aspartate, tivortin, pathological morpho-functional disintegration

The work is fragments of the research project "Development of new therapeutic and prophylactic methods and pathogenetic background of their use in inflammatory periodontal diseases together with metabolic syndrome", state registration No0115U0021970.

Treatment of patients with liver cirrhosis (LC) and its complications is one of the most difficult problems of surgery, in particular, surgical hepatology and biliary surgery [10, 6]. According to the WHO, the frequency of LC is steadily increasing [12, 14]. According to the results of pathological studies this data differs from 1 to 11% [9, 15]. High LC incidence is due to increased incidence of acute viral hepatitis, especially, type B, C, D, resulting in the hepatic parenchymal inflammatory-destructive process transformation into chronic [2, 3, 8] with LC formation and other complications development [11]. Liver cirrhosis is accompanied by high mortality, entering, according to the WHO, up to 10 diseases with the highest mortality [7].

To investigate the hepatic morpho-functional changes we created an experimental model of LC. The blood serum and erythrocytes, hepatic and pancreatic parenchyma oxidative-antioxidant homeostasis activity as well as structural changes in liver in conditions of LC were studied. Taking into account systemic disorders in patients with LC the rate of hepatocellular insufficiency progression, as well as the frequent occurrence of multiorgan failure syndrome with pancreatic, gallbladder, stomach lesions and vascular component in this disease involvement into the pathologic process we suspected the influence of one of the typical pathological processes – inflammation – in the pathogenesis of these disease. Lipoperoxidation activation is one of the body's response manifestations to the alterative factors action that initiate a systemic inflammatory response.

The purpose of the study is to investigate the LC pathophysiological mechanisms in rats and to investigate the efficacy of pathogenetical correction of these pathology using L-arginine aspartate and tivortin.

Materials and methods. The experiments were performed under conditions of chronic experiment on 180 male rats lines Wistar adult (over 6 months), body weight of (200±20) g, kept in conventional vivarium conditions. Using random sampling, the animals were divided into following groups: 1 group – animals without simulated pathology (intact, n=10), which via a plastic probe were intragastrically injected 4 ml of solvent (refined sunflower oil); group 2 – animals with simulated LC (n=80), which is a pathological condition was reproduced by introducing hepatotropic poison – carbon tetrachloride, which produces a direct cytolytic effect on the liver parenchyma. A solution was prepared from pure (99.99 %) of the drug by the addition of refined sunflower oil in the ratio 1:1 and was administered intragastrically using a plastic probe twice a week (Monday and Thursday) in a volume of 4 ml for 10 weeks. Control of the formation of experimental liver cirrhosis was carried out by diagnostic laparotomy with biopsy and subsequent histological examination of the biopsy specimens; 3 group – rats (n=45) in which LC was modeled and L-Arginine aspartate was performed (50 mg/kg, intraperitoneally [i.p.] daily during the whole period of investigation; group 4 – rats (n=45) in which LC was modeled and pharmacological correction using tivortin (100 mg/kg, i.p.) was carried out once daily during the whole period of investigation with biopsy and subsequent histological examination of the biopsy specimens.

During the period of experience 21 animals died from acute liver failure, which was 26.3 % of the total number of animals that modeled LC. Left 59 rats which after 12 h, 1, 3, 5 and 7 days after completing the simulation of the LC were taken from experiment by Thiopental sodium (100 mg/kg, i.p.) overdose. After euthanasia the blood and internal organs of rats were sent for biochemical study.

In a separate series of studies, after LC simulation in 90 rats euthanasia was performed after 1, 6, 12 h, 1, 3, 5 and 7 days. Blood was taken from animals (each group in a certain period of time consisted out of 6 rats) [8] and the concentration of intermediate products of lipid peroxidation – Malondialdehyde (MDA), Conjugated Dienes (CD) – and activity of antioxidant enzymes – Superoxidedismutase (SOD), Glutathioneperoxidase, Glutathionereductase – was determined by standard methods.

After euthanasia, the liver and pancreas were taken from all animals, tissue samples were homogenized in the environment of 10 mmol Tris-HCl buffer (pH=7,4) at a ratio of 1:9. To obtain a solid fraction, the homogenate was centrifuged for 10 min at a speed of 3000 g ($t = 0-2^{\circ}\text{C}$). Supernatant was used to determine the concentration of MDA, CD and the activity of antioxidant enzymes – SOD, Glutathione peroxidase and Glutathione reductase. The intermediate products of lipoperoxidation concentration were determined by standard methods. SOD activity was determined by the level of inhibition of Nitro blue tetrazolium recovery in the presence of Nicotinamide adenine dinucleotide (NADH) and Phenazine methosulfate; the activity of Glutathione peroxidase – by the velocity of oxidation of glutathione in the presence of tert-Butyl hydroperoxide; the activity of Nicotinamide adenine dinucleotide phosphate (NADPH) – Glutathionereductase – by the velocity of recovery oxygenated Glutathione in the presence NADPH, the concentration of total Glutathione - by the method described in work [5], the content of α -tocopherol - by the method [5].

To implement histological study the tissue material was fixed in 10 % solution of neutral buffered formalin. Further histological preparations were performed according to standard techniques [1]. Production of serial paraffin sections with a thickness of 4–6 μm were carried out on the sliding microtome. Staining preparations were made with hematoxylin and eosin.

For document images of histological preparations were taken on the computer monitor using the Delta Optical microscope and digital camera (Digital Camera SCMOS) using software ToupView at different magnifications.

Biochemical and morphological studies were performed in rats with LC, as well as rats were administered L-Arginine aspartate (50 mg/kg, i.p.) and tivortin (100 mg/kg, i.p.) for medicinal purposes which are characterized by antiinflammatory (due to their antioxidant, membrane stabilizing, energy saving and other effects) and hepatoprotective properties.

The results were processed statistically using one-way ANOVA parametric criteria. $p < 0.05$ was chosen as the minimal criteria of reliability.

Results of the study and their discussion. In the study of the liver structure in animals with experimental LC it was found that the structure of hepatic lobules was impaired. Central veins were well visualized, their lumens were slightly expanded, contained a small amount of red blood cells. In most cases, the lumens of sinusoidal capillaries were not detected, or were revealed only in centrolobular position. Organization of hepatic lamina was impaired in whole lobule. The cytoplasm of hepatocytes located in centrolobular zone, the middle third of the lobule and periportally fields were changed, and the vast majority of hepatocytes were light and empty, which is the sign of ballooning degeneration. The majority of hepatocytes contained nuclei, however, they are both visually and morphometrically decreased and shrunk.

In individual cells on the background of destructive changes of the cytoplasm nuclei were with signs of caryopycnosis and caryolysis, indicating the presence of dystrophic-necrotic manifestations. The contours of the cells changed dramatically, the vast majority increased, deformed, cell-to-cell junctions were damaged. Portal tracts expanded mainly due to the expansion and plethora of vessels, mucoid and fibrinoid swelling of blood vessels walls, perivascular edema and lymphoplasmacytic infiltration, the formation of septal sclerosis (fig. 1), a minor expansion of the bile duct without visualization of bile pigments.

Histological examination of the liver of animals with a simulated LC in the correction by L-Arginine aspartate it was found that partial structure of the liver parenchyma was significantly restored. Central veins were well visualized, with their lumens slightly expanded, remained moderately plethoric. Their lumens were detected signs of venous congestion. Sinusoidal capillaries are moderately expanded. Organization of hepatic lamina were restored in whole lobule. The cytoplasm of the cells was structured. The vast majority of hepatocytes contained nuclei, the relative amount of damaged cells was decreased. Cell-to-cell junctions in the vast majority of cells were recovered.

The regenerative activity of the tissues visually increased. Portal tracts remained expanded, mainly due to the expansion and plethora of blood vessels, a slight expansion of the bile ducts, moderate lymphohistiocytic perivascular infiltration with marked stromal collagenization. Perivascular edema remained minor (fig. 2).

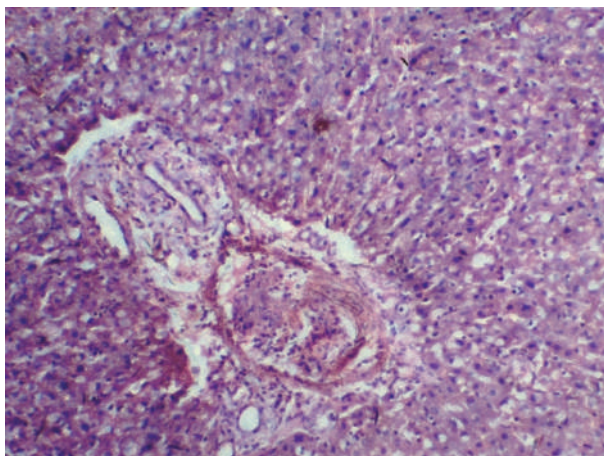


Fig. 1. The structure of the liver of animals with simulated LC. A significant expansion of portal tracts with the formation of septal sclerosis, perivascular edema and lymphoplasmacytic infiltration. Dystrophic changes in hepatocytes. Staining with hematoxylin and eosin. $\times 100$.

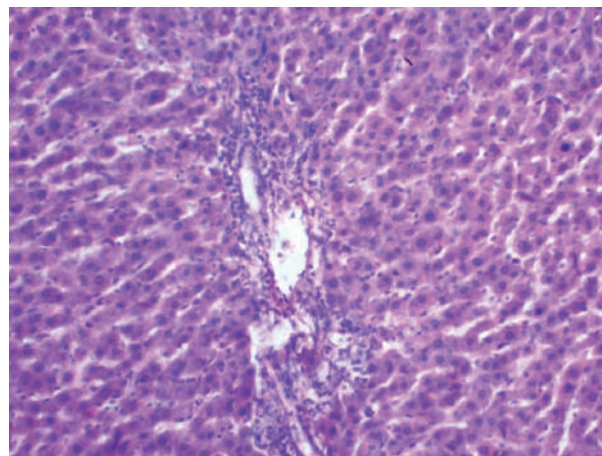


Fig. 2. The structure of the liver of animals with LC using L-Arginine Aspartate for the pharmacological correction. Expansion of portal tracts mainly due to the expansion of blood vessels and lymphoplasmacytic infiltration. Reduction of dystrophic changes in hepatocytes. Staining with hematoxylin and eosin. $\times 100$.

In animals with experimental LC after the introduction of Tivortin, the same with the introduction of L-Arginine aspartate, but more pronounced, normalization of the liver morphological structure and intraorganic vascular network was observed.

In the blood of rats with experimental LC significant accumulation of MDA and CD was noted, the absolute concentration of these substances after 12 hours of the experience was, respectively, 2.69 ± 0.18 nmol/l and 0.70 ± 0.07 μ mol/l, which is 1.9 times ($p < 0.001$) and 1.7 times ($p < 0.01$) exceeded the relevant figures in the control group (table 1).

Table 1

Concentrations of lipoperoxidative products and activity of antioxidant enzymes in blood of rats at different times after the LC simulation

Index	Control group (n=9)	The value of an index at the time of observation (M \pm m)				
		12 hours	1 day	3 days	5 days	7 days
MDA, nmol/l	1,41 \pm 0,11	2,69 \pm 0,18***	3,77 \pm 0,29***	4,41 \pm 0,37***	3,86 \pm 0,26***	2,27 \pm 0,23**
CD, μ mol/l	0,41 \pm 0,05	0,70 \pm 0,07**	0,86 \pm 0,08***	0,97 \pm 0,11***	0,84 \pm 0,07**	0,67 \pm 0,06*
Catalase, i.u.	1,92 \pm 0,13	1,31 \pm 0,13**	1,18 \pm 0,12***	1,08 \pm 0,10***	1,21 \pm 0,11**	1,49 \pm 0,14*
SOD, U/ml	2,79 \pm 0,17	1,68 \pm 0,16**	1,56 \pm 0,14***	1,48 \pm 0,13***	1,62 \pm 0,17**	1,97 \pm 0,20*
Total gluta-thione, μ mol	20,1 \pm 0,6	15,7 \pm 1,1*	15,1 \pm 1,0**	14,4 \pm 1,2**	15,6 \pm 1,3*	16,6 \pm 1,3
α -tocopherol, μ mol/ml	51,8 \pm 3,7	38,9 \pm 3,8*	35,9 \pm 3,5**	33,4 \pm 3,3**	36,2 \pm 3,7*	37,3 \pm 3,6*

Notes: The difference of indexes compared to those in the control group are statistically reliable: * $p < 0.05$, ** – $p < 0.01$, *** $p < 0.001$ (one-way ANOVA criteria).

In the future, the levels of MDA and CD continued to increase, reaching on the third day of the pathological process, when the value of indices in 3.1 times and 2.4 times higher than in control ($p < 0.001$). Further, a slight decrease in the level of MDA and CD was found, however, on the seventh day it exceeded that in control ($p < 0.05$).

Under these conditions in the blood of rats a significant decrease in the activity of antioxidant enzymes – catalase, SOD, Glutathione, α -tocopherol was observed, which was the minimum for 3 days after LC simulate ($p < 0.01$). The activity of the enzymes has not recovered to the 7-day experiment ($p < 0.05$).

The concentration of liporexidation intermediate products in erythrocytes in the 1st – 5th day of LC was the same, maximal MDA and CD concentration was registered on the 3rd day when these indexes were 2,5 times ($p < 0.001$) higher pertaining the same in the control group. The activity of catalase, SOD, Glutathioneperoxidase and Glutathionereductase were maximally reduced on the 3rd day of LC ($p < 0.05$).

In the LC rat's liver tissue it was observed that a significant increase in the level of MDA and CD, which is already in 12 hours after simulate of the pathological process, respectively, on 85 and 129 % was higher than in the control group ($p < 0.001$, Table 2). The maximum marked accumulation of intermediate

lipoperoxidative products was observed in the first day ($p<0.001$) with a slight decrease in the indices on the 3rd ($p<0.001$) and 5th ($p<0.05$) days of the trial. On the 7th day the indices were not different in the main and control groups ($p>0.05$). The course of the pathological process is also characterized by a pronounced ($p<0.05$) decrease of the activity of antioxidant enzymes, which lasted to the 5th day of experiment (table 2).

Table 2

Concentrations of lipoperoxidative products and activity of antioxidant enzymes in liver parenchyma of rats at different after the LC simulation

Index	Control group (n=9)	The value of an index at the time of observation (M±m)				
		12 hours	1 day	3 days	5 days	7 days
MDA, $\mu\text{mol/g}$	2,82±0,23	5,21±0,41***	6,43±0,51***	5,49±0,42***	4,87±0,31***	2,82±0,23
CD, $\mu\text{mol/g}$	0,41±0,06	0,94±0,09***	1,12±0,10***	1,06±0,10***	0,88±0,08*	0,41±0,06
SOD, U/g	1,86±0,17	1,07±0,07**	1,03±0,07***	1,00±0,06***	1,14±0,09*	1,86±0,17
Glutathione peroxidase, U/g	2,56±0,21	1,34±0,13**	1,21±0,11***	1,29±0,11**	1,49±0,12*	2,56±0,21
Glutathione reductase, U/g	2,66±0,13	1,62±0,14**	1,32±0,11***	1,41±0,12***	1,78±0,14*	2,66±0,13

Notes: The difference of indexes compared to those in the control group are statistically reliable: * $p<0.05$, ** – $p<0.01$, *** $p<0.001$ (one-way ANOVA criteria).

Similar changes, in particular, the decrease of the activity was noted about antioxidant enzymes in pancreatic parenchyma – SOD, Glutathione peroxidase and Glutathione reductase ($p<0.05$). The course of LC under these conditions was accompanied by a significant ($p<0.05$) increase of the level of MDA and CD, and decrease of the activity of antioxidant enzymes in the parenchyma of the pancreas.

Pharmacological correction of experimental LC using L–Arginine aspartate and Tivortin was a significant decrease of the concentration of intermediate lipoperoxidative products and activation under the influence of the applied compounds with enzymatic antiradical activity.

The results after critical analysis allow to formulate the following basic provisions relating to the pathophysiological mechanisms of experimental LC. Firstly, LC is accompanied by increased activity of lipid peroxidation processes, which is manifested by accumulation of intermediate lipoperoxidative products and decreased activity of enzymatic and non-enzymatic branches of the system of antioxidant protection. These facts are negotiated with the opinion of prominent experts [3, 4, 10] about the pathogenetic role of intensification of lipid peroxidation process in some pathological processes, in particular, inflammation, high temperatures, radiation factors, other damaging influences.

The data indicate the participation of the blood cellular apparatus, namely red blood cells, in the pathogenetic mechanisms of hepatocytes death, because in the erythrocytes the data were unidirectional with those in the blood plasma. i.e. the increase in the concentration of lipoperoxidative products and a decrease in the activity of antioxidant enzymes. Summarizing these results and suggestions one could suppose the generalization of the pathological process with LC which explains how the speed of his progression and spread of abnormal changes in the cells that should be taken into account in the clinical conditions in determining the tactics of patients treatment [2, 14].

We identified the associated processes of lipid peroxidation intensification and the antioxidant defense system inhibition occurring directly in the tissues of the liver. These data explain the speed of disease progression, high volume and typically of irreversibility of the pathological process of cells destruction in case of LC [7, 14]. According to anatomic unity, common physiological functioning and similar to those in liver parenchyma disorders which were manifested by the shift of the dynamic equilibrium in the system “lipid peroxidation – the antioxidant system” in the direction of the intensification of processes of lipid peroxidation, it was clearly demonstrated somewhat less pronounced than in the liver tissue, lipoperoxidative products accumulation and antioxidant defense system inhibition in the pancreatic parenchyma.

Comparable to the results of biochemical research are the data of morphological studies, which confirm a unified concept of the formation of pathological morpho–functional disintegration in conditions of formed pathology [3]. Analyzing the data become apparent pathophysiological mechanisms of multiple organ failure syndrome with LC, hepatic fibrosis, portal hypertension, liver failure. Given the morphological disorders and the intensification of lipid peroxidation processes and the associated inhibition of the activity of antiradical protection, important in terms of planning schemes of LC complex pathogenetically treatment is the inclusion of drugs with antioxidant properties that will help reduce the severity of hepatocytes destruction to prevent liver failure.

We consider, useful in the future, further implementation of our research results, which indicate a normalizing effect of L–Arginine aspartate and Tivortin on morpho–functional activity in experimental LC. Their hepatoprotective activity was similar in severity with a slight predominance of that of L–Arginine Aspartate and manifested, beginning with the 6th hour after LC was simulated.

Conclusions

1. The LC manifestation is accompanied by lipid peroxidation processes activation in the blood, erythrocytes, liver parenchyma and the pancreas which is accompanied by intermediate lipoperoxidative products accumulation and both antioxidant system enzymatic and non-enzymatic branches activity inhibition.

2. Such way of pathological process manifestation explains the speed of his progression, high expansion and irreversible destruction of cells with LC that from fundamental view demonstrates the unity of pathogenetic mechanisms of damage of the liver parenchyma under conditions of the studied pathology, reflects the systemic inflammation in LC, highlights the formation of pathological morpho-functional disintegration, which is the basis for the development of multiple organ failure syndrome.

3. L-Arginine aspartate and Tivortin administration in experimental LC contributes to lipid peroxidation processes suppression in blood, erythrocytes and in both hepatic and pancreatic parenchyma and normalizes the functioning of liver cells. Therefore one should suppose these data as the experimental background of these pharmacons clinical efficacy testing.

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Реферати

**ПАТОЛОГІЧНА МОРФО-ФУНКЦІОНАЛЬНА
ДЕЗІНТЕГРАЦІЯ ЯК ПРОВІДНИЙ
ПАТОГЕНЕТИЧНИЙ МЕХАНІЗМ ПРИ
ЕКСПЕРИМЕНТАЛЬНОМУ ЦИРОЗІ ПЕЧІНКИ**

**Вастьянов Р.С., Дзыгал О.Ф., Горлицина О.А.,
Михайленко В.Л., Лапшин Д.Є., Назаренко О.Я.**

Мета дослідження - вивчення патофізіологічних механізмів цирозу печінки у щурів та дослідження ефективності патогенетичної корекції цієї патології застосуванням L-аргініну аспартату та тивортину. Проведені експериментальні дослідження підтвердили залучення процесів ліпопероксидації до патогенетичних механізмів цирозу печінки. Результати свідчать про участь клітинного апарату крові в патогенетичних механізмах загибелі гепатоцитів. Співставними з результатами біохімічних досліджень є дані морфологічних досліджень, які підтверджують єдину концепцію формування патологічної морфо-функціональної дезінтеграції за умов сформованої патології, що є підставою розвитку синдрому

**ПАТОЛОГИЧЕСКАЯ МОРФО-ФУНКЦИОНАЛЬНАЯ
ДЕЗИНТЕГРАЦИЯ КАК ВЕДУЩИЙ
ПАТОГЕНЕТИЧЕСКИЙ МЕХАНИЗМ ПРИ
ЭКСПЕРИМЕНТАЛЬНОМ ЦИРРОЗЕ ПЕЧЕНИ**

**Вастьянов Р.С., Дзыгал А.Ф., Горлицына А.А.,
Михайленко В.Л., Лапшин Д.Е., Назаренко О.Я.**

Цель исследования - изучение патофизиологических механизмов цирроза печени у крыс и исследование эффективности патогенетической коррекции данной патологии применением L-аргинина аспартата и тивортина. Проведенные экспериментальные исследования подтвердили вовлечение процессов липопероксидации в патогенетические механизмы цирроза печени. Результаты свидетельствуют об участии клеточного аппарата крови в патогенетических механизмах гибели гепатоцитов. Сопоставимыми с результатами биохимических исследований являются данные морфологических исследований, подтверждающие единую концепцию формирования патологической морфо-функціональної дезінтеграції в умовах воспроизводимой патологии, что является основой развития синдрома

полиорганной недостаточности. Авторы утверждают, что позитивні результати застосування L-аргініну аспартату та тивортину за умов експериментального цирозу печінки є експериментальним обґрунтуванням доцільності тестування клінічних ефектів вказаних лікарських сполук.

Ключові слова: експериментальний цирроз печінки, перекисне окислення ліпідів, морфологічні порушення, патофізіологічні механізми, L-аргініну аспартат, тивортин, патологічна морфо-функціональна дезінтеграція

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полиорганной недостаточности. Авторы утверждают, что положительные результаты применения L-аргинина аспартата и тивортина при экспериментальном циррозе печени является экспериментальным обоснованием целесообразности тестирования клинических эффектов указанных лекарственных препаратов.

Ключевые слова: экспериментальный цирроз печени, перекисное окисление липидов, морфологические нарушения, патофизиологические механизмы, L-аргинина аспартат, тивортин, патологическая морфо-функциональная дезинтеграция

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DYNAMICS OF ATP-POSITIVE DENDRITIC CELLS IN RAT'S OROPHARYNGEAL SUBMUCOSA AFTER ANTENATAL ANTIGEN ADMINISTRATION

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Purpose of the work was to establish dynamics and morphology of DCs, located in oropharyngeal submucosa the postnatal period after antenatal antigen effect on a fetus. DCs were detected on the cryostat sections of the pharynx tissue by using the Vakshtein-Meizel method. In experimental newborns, the DCs absolute number was found to be greater than in the control and did not change during the first week, unlike in the control, where this index did not change significantly over the two weeks of life. All groups of animals have been increased DCs absolute number by third week of life, while the antigen load on the body increases. Experimental animals, regardless of the antigen administration mode, have been taken place DCs activation earlier than in control, that is, at 7th life day. Animals which underwent antenatal antigen administration during fetal period has been increased number of their processes compared to control. Although it was founded that DCs in experimental groups are stained more shade than in control group, which indicating a more active ATP accumulation.

Key words: ATP, antenatal antigen administration, dendritic cell, pharynx, local immunity.

This work is a fragment of the research project "Features of the rat's organs structure under the influence of different factors during the pre- and postnatal periods", state registration No. 0120U103118.

Dendritic cells (DCs) form a widely distributed cellular net throughout the body. DCs not only exert immune-surveillance for antigens of different origin, but also later activates naive T lymphocytes by giving rise to various immunological responses [2]. The immune complex of oral cavity and pharynx (as a part of a MALT – Mucosa Associated Immune Tissue) might represent the deserve immunological challenges continuously faced by its mucosa. DCs take a crucial part in linking innate and adaptive immunity, either as in mediating immunity or tolerance. Mucosa associated DCs, especially of oral mucosa, should be thoroughly studied in our attempt to understand formation oral immunity. Besides, it is not always possible to extrapolate oral DCs function from their counterparts in non-oral tissues [7]. Also mucus form a nonspecific physical barrier and constrains the immunogenicity of antigens by delivering tolerogenic signals [13].

It is proved that antigen-presenting cells play a central role in transferring information from the periphery of the organism to lymphoid organs. They deliver important signals which result in T cell unresponsiveness with antigen-specific tolerance induction. The initiation of effector CD8⁺ T-cell responses needs the presentation of peptide bond derived from internalized antigen on class I major histocompatibility complex molecules by DCs in a process called cross-presentation [4].

Antigen load on body, especially on barrier mucosa, can be materialize not only bacteria and viruses but artificially by vaccination, or by antenatal antigen administration on fetus in case mother has undergone some infection during pregnancy [5]. According to Apostolopulose's opinion, a major aim in vaccine development is to induce powerful, specific T-cell responses [1]. This is achieved by targeting antigen to cell surface molecules on DCs that begins receptor mediated endocytosis for loading onto MHC molecules and stimulation of T-cell responses.

It is known, that type III interferon (IFN-λ) is important for innate immune protection at mucosal surfaces and has therapeutic benefit against influenza A virus infection (IAV). According to Hemann's opinion, IFN-λ signaling in DCs populations was critical for the development of protective IAV-specific CD8⁺T cell responses. It is proofed that mice lacking the IFN-λ receptor had decreased CD8⁺ T cell

responses relative to wild type and exhibited reduced survival after IAV re-challenge. Analysis of DCs revealed IFN- λ signaling directed the migration and function of CD103⁺ DCs for development of optimal antiviral CD8⁺ T cell responses. Thus, IFN- λ serves a critical role in bridging innate and adaptive immunity from mucosa to lymph nodes to program DCs to direct effective T cell immunity against IAV [6].

Adenosine also signaling increased IL-10 secretion while decreasing IL-12p40 secretion in human monocyte-derived DCs [9]. Actually, Antigen DCs are one of the primary targets for adenosine to suppress T and NK cell responses [3, 10].

DCs are an important component of the MALT system. The content of adenosine represents the functional activity of dendritic cells and changes throughout life, as well as with increasing antigen administration. Adenosine content dynamics in the cytoplasm of dendritic cells after antenatal antigen administration on a fetus has not been studied. Determination of adenosine accumulation features will help to track the reactivity of dendritic cells in response to antigen administration and to reveal formation mechanisms of the oral immune system, especially for children, which mothers have undergone antigen loading during pregnancy, that will form the basis for the formation of new pediatric approaches to care for at-risk group children.

The purpose of the work was to establish dynamics and morphology of DCs, located in oropharyngeal submucosa, at the postnatal period after antenatal antigen administration on a fetus.

Materials and methods. Pharynges of the 124 white laboratory rats were taken as an object of the study. Oropharynges were taken for examination at 1, 7, 14, 21, 45 days of postnatal life. Animals were divided into four groups: I – intact animals, which were born from healthy rats without any antigen administration during pregnancy, II – animals, which were exposed to antenatal antigen administration at 18th day of prenatal development with the method of Voloshyn M.A. (2010), III – animals which were exposed to amniotic fluid antigen administration at the 18th day of prenatal development with the method of Voloshyn M. A. (2011), IV – control animals, which were exposed to antenatal intrafetal injection of saline solution on the 18th day of prenatal development. Rats were born full term and absolutely healthy. It is said that all animals with any symptoms of a disease were avoided to take at experiment. Control group of animals was used for proofing that a process of operating got no effect on a fetus, but antigen leading does. Sex differences were not considered. As antigen have been used split virus inactivated Influenza vaccine Vaxigrip. DCs were detected on the cryostat sections of the pharynx tissue by using the Vakhshstein-Meizel method. Instead of more type of lymphocytes, DCs have high activity of ATPase, because of the activity of the ATP-dependent proton pump depends on the gradual decrease of pH in the endosomes and lysosomes, activation of proteases in the endocytosis of antigens. Other pharyngeal cells exhibit moderate to low activity of ATPase. Control of the reaction was carried out with histological samples rich in ATPase. Samples were embedded in glycerol-gelatin. The absolute number of DCs and its dendrites was counted in a oropharyngeal submucosa on a unit area of 15000 μm^2 using a microscope with oil immersion technique (x630). The variation statistics methods via program «STATISTICA 6.1» (StatSoft Inc., № AXXR 712D833214FAN5) was used to compare differences in number of DCs and DCs dendrites. The $p \leq 0.05$ were considered significant. Supporting and withdrawal of animals from experiment was carried out in accordance with the requirements of the European Commission Directive (86/609/EEC), Law of Ukraine № 1759-VI (15.12.2009) On the Protection of Animals from Cruelty.

Results of the study and their discussion. In newborn intact animals ATP-positive DCs are found in the submucosa of oral part of the pharynx, mainly under the basement membrane. The largest number of ATP contains in the cytoplasm, and provides membrane processes and energy metabolism. Cell bodies are of triangular elongated shape, rough contours of the plasma membrane with brown deposits in the cytoplasm. The nucleus is light, elongated, with wavy contours. The dendrites are spatially oriented mainly along the basement membrane and posses button-shaped endings. In experiment and control there is no difference between topography of DCs and their dendrites.

At the 1st day of life in the number of DCs oropharyngeal submucosa reaches 1.2 ± 0.65 at 15000 μm^2 (Fig.1) and the number of its dendrites is 2.7 ± 0.03 at 15000 μm^2 (fig.2). In experimental animals of the same life term dendrites number increases statistically up to 4.1 ± 0.03 at 15000 μm^2 and 3.9 ± 0.18 at 15000 μm^2 in the animals of the second and third groups compared to control animals. The difference in DCs number in oropharyngeal submucosa is not statistically verified between experimental and control animals (fig.1).

At the seventh day of life in experimental animals there is a tendency of a higher content of DCs number compared to control animals. The number of DCs dendrites in the experimental animals is statistically significantly higher than in control. At describing period in all group animals number of ATP-positive DCs and their dendrites has a tendency to increase compared to the previous observation period (fig. 1, 2).

At the fourteenth day of life DCs are located in the oropharyngeal submucosa beneath the basement membrane, their processes are located parallel to the basement membrane. Their number is statistically significantly increased from 7th days of life to 3.2 ± 0.15 per $15000 \mu\text{m}^2$. The DCs number in the experimental oropharyngeal submucosa tends to increase compared to control. In intact animals, the accumulation of ATP-positive material in the cytoplasm increases, proved by the darker staining of DCs. Statistically significantly increasing of dendrites number, compared to the previous observation period, is also revealed. However the length is reduced. Among other common findings we established are fan-shaped dendrites in experiment instead of button-shaped dendrites in control group (Fig.1, Fig.2). After antenatal antigen administration, DCs are stained more vividly compared to animals in control group. In experimental animals of both groups, DCs are characterized by greater number of dendrites than in control. DCs dendrites of experimental animals are visually thicker and more intense in color than in the animals of control group.

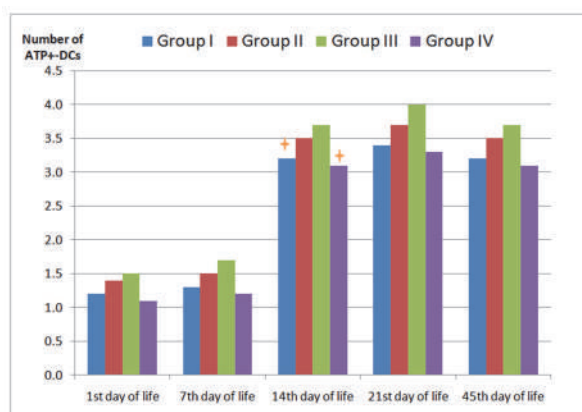


Fig.1. ATP⁺-DCs' Dynamics in Rats' Oropharyngeal Submucosa on the Unit Area ($15000 \mu\text{m}^2$, Vakhshstein-Meizel method).

Notes: the symbol \star means that the result is statistically significant in relation to the previous observation period.

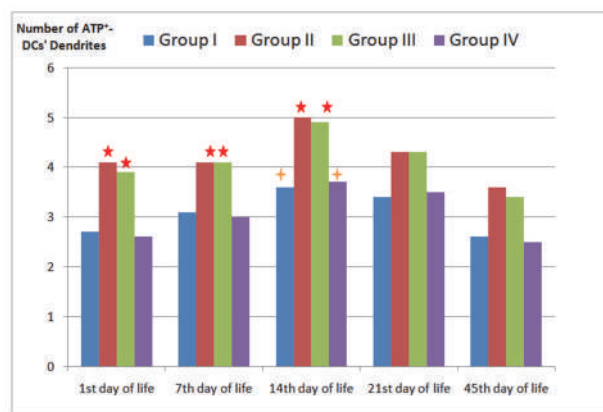


Fig.2. Dynamics of ATP⁺-DCs' Dendrites in Rats' Oropharyngeal Submucosa on the Unit Area ($15000 \mu\text{m}^2$, Vakhshstein-Meizel method). Notes: the symbol \star means that the result is statistically significant with respect to the control group, the symbol \star means that the result is statistically significant in relation to the previous observation period.

Intensive deposition of ATP-positive material contents of intracytoplasmic inclusions of DCs persists from the twenty-first up to the forty-fifth day of life in the animals of control and intact group, the dendrites of DCs are predominantly fan-shaped. The number of them is at the same level with appropriate index of the previous observation period. In animals which underwent antenatal antigen administration during fetal period there are cells with more short predominantly fan-shaped dendrites, compared to animals of control group. At the 21 day of life there is a tendency to increase the DCs number in experimental animals, compared to control ones.

DCs number increases by 14th day of life in all groups of animals, while the antigen load on the body increases. Terms of increasing antigen load on body coincides with terms of changing type of feeding food by getting supplements (solid food) and can be explained by that. This period takes time from the 14th day up to the 21th day of postnatal life. In experimental animals, regardless of the mode of antigen administration, DCs activation takes place earlier than in animals of the control and intact groups, that is, at 7th day of life. In animals which underwent antenatal antigen effect during fetal period the DCs number does not change in comparing to animals of the control and intact groups. However, the number of their processes overgoes control and intact ones. Obtained results coordinate with our previous results [8], also statistically significantly differences between number of DCs in submucosa of nasopharynx and oropharynx was not found.

DCs initiate and modulate primary immune responses by attracting and activating naive T cells. They are able to coordinate tolerance or immune response depending on their activation status, that is why DCs are also considered as "orchestrating" cells of the immune response [12, 15].

It is settled, that antenatal antigen administration leads to acceleration of the release of immunologically immature PNA⁺-lymphocytes emerges from thymus to the peripheral immune organs, including pharyngeal wall as MALT-representative. According to the concept of "Lymphocyte – morphogenesis factor" PNA⁺-lymphocytes influence on the morphogenesis of surrounding cells, changing intercellular and cellular matrix interaction. Microenvironment takes crucial point in differentiation of functional DCs [11]. Antigen presenting cells, such as DCs, are one of the primary targets for adenosine to suppress T and NK cell responses. Despite not enough understanding of molecular mechanisms of adenosine regulation of DCs it is founded that adenosine receptor stimulation strongly suppresses DCs

activation [3]. Cytokine expression profile of Adenosine-differentiated DCs is deeply altered compared to classic myeloid DCs, as well as it is characterized by a mix of proinflammatory and anti-inflammatory cytokines and up-regulated by immune suppressor and tolerogenic factors.

High activity of ATPase is explained by activation of proteases in the process of antigen endocytosis. On one hand, adenosine is a well-studied neurotransmitter, but on the other hand as a part of ATP it also exerts deep immune regulatory functions. While ATP stimulates immune responses by exact inflammasome activation, its degradation product adenosine acts rather anti-inflammatory. In this case it decreases regulation of DCs function and dampens T cell activation and cytokine secretion. DC derived adenosine can also act back onto the DCs in an autocrine manner. As a result DCs functions that are normally involved in stimulating immune responses are suppressed [14]. This likely can lead to depressed reactions of local immunity.

Conclusion

Activation and increasing of DCs number in oropharyngeal submucosa of control animals is observed at the first and fourteenth days of life. In experimental animals, regardless of the method of exposing antigen, the second wave of DCs activation takes place earlier than in control, that is a 7th day of life. Animals which were exposed to antenatal antigen administration have been increased dendrites number compared to control. In experimental groups it was founded that DCs are stained more shade than in control group, which indicating a more active ATP accumulation.

Prospects for the further research lie in the fact that it is planned to study the quantitative content of ATP in DCs cytoplasm after antenatal antigen administration.

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Реферати

ДИНАМІКА АТФ-ПОЗИТИВНИХ ДЕНДРИТНИХ КЛІТИН В ПІДСЛИЗОВІЙ ОСНОВІ РОТОВОЇ ЧАСТИНИ ГЛОТКИ ЩУРІВ ПІСЛЯ ВНУТРІШНЬОУТРОБНОГО ВВЕДЕННЯ АНТИГЕНА

Григор'єва О.А., Матвейшина Т.М., Тополенко Т.А.

Метою дослідження було встановити динаміку та морфологію дендритних клітин підслизової основи ротової частини глотки щурів після внутрішньоутробного введення антигена. Дендритні

ДИНАМИКА АТФ-ПОЛОЖИТЕЛЬНЫХ ДЕНДРИТНЫХ КЛЕТОК В ПОДСЛИЗИСТОЙ ОСНОВЕ РОТОВОЙ ЧАСТИ ГЛОТКИ КРЫС ПОСЛЕ ВНУТРИУТРОБНОГО ВВЕДЕНИЯ АНТИГЕНА

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Целью исследования было установить динамику и морфологию дендритных клеток подслизистой основы ротовой части глотки крыс после внутриутробного введения антигена. Дендритные клетки были выявлены на криостатных

клітини були виявлені на криостатних зрізах тканини глотки за допомогою методу Вахштейна-Мейзеля. Встановлено, що у новонароджених експериментальних тварин абсолютна кількість дендритних клітин більша, ніж в контролі, та не змінюється протягом першого тижня, на відміну від контролю, де цей показник значно не змінюється протягом двох тижнів життя. У всіх груп тварин абсолютна кількість дендритних клітин збільшується протягом третього тижня життя, одночасно зі збільшенням антигенного навантаження на організм. В експерименті, незалежно від способу введення антигену, активація дендритних клітин відбувається раніше, ніж у контролі, тобто на 7 добу життя. У експериментальних тварин, порівняно з контролем, збільшується кількість відростків дендритних клітин, а також самі дендритні клітини забарвлені темніше, ніж у контролі, що свідчить про більш активне накопичення АТФ.

Ключові слова: АТФ, внутрішньоутробне введення антигену, дендритні клітини, глотка,
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срезах ткани глотки с помощью метода Вахштейна-Мейзеля. Установлено, что у новорожденных экспериментальных животных абсолютное количество дендритных клеток больше, по сравнению с контролем, на протяжении первой недели жизни и не изменяется, в отличие от контроля, где этот показатель значительно не изменяется в течение двух недель. У животных всех исследуемых групп абсолютное количество дендритных клеток увеличивается на протяжении третьей недели жизни, одновременно с увеличением антигенной нагрузки на организм. В эксперименте, активация дендритных клеток происходит раньше, чем в контроле, то есть на 7 сутки жизни. У экспериментальных животных, по сравнению с контролем, увеличивается количество отростков дендритных клеток. У экспериментальных животных увеличивается количество дендритов по сравнению с контролем, а также установлено, что сами дендритные клетки окрашены темнее, чем в контроле, что свидетельствует о более активном накоплении АТФ.

Ключевые слова: АТФ, внутриутробное введение антигена, дендритные клетки, глотка, местный иммунитет.
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ORGANOTOXIC EFFECT OF SINGLE INTRATRACHEAL ADMINISTRATION OF LEAD NANOPARTICLES OF DIFFERENT SIZES

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Cardiovasotoxic effect of a single intratracheal administration of lead nanoparticles of different sizes was studied in the experiment. Colloidal solutions of lead sulfide (PbS in sodium polyphosphate) with an average size of 26–34 nm and 50–80 nm, and lead nitrate Pb(NO₃)₂ in ionic form which is well soluble in water were used. Toxic effects were assessed 12 days after exposure. Morphological changes were found in the myocardium and to a greater extent in the atria. Mild disorders, such as enlarged interstitial space and dystrophic changes of individual cardiomyocytes were found after administration of PbS_{26–34nm} and PbS_{50–80nm} nanoparticles. Thus, with intratracheal administration of lead, the toxic effect of nanoparticles PbS_{26–34nm} and PbS_{50–80nm} was manifested more compared with an effect caused by the action of the ionic form of lead Pb(NO₃)₂. The toxic effect of lead nanoparticles was mainly evident in the atrial myocardium cardiomyocytes, while the aortic wall remained almost unaffected. The most pronounced structural changes were observed in the lungs and bronchi, which may be due to the route of the toxicant administration.

Key words: lead, nanoparticles, intratracheal intoxication, morphological changes, myocardium, aorta, lungs.

The work is a fragment of the research projects "Investigation of cardiovasotoxic action mechanisms of heavy metal nanoparticles (on the problem of biosafety of nanomaterials)", state registration No. 0119U100182; "Changes in internal organs and regulatory systems under the conditions of experimental damage and historical aspects of histology, cytology and embryology development in Ukraine", state registration No. 0116U000121 and "Study the of tissue reaction features and their modulation in lesions of various origins", state registration No. 0120U102691.

New physicochemical properties of the engineered nanoparticles make them very attractive for industrial and biomedical use. Nowadays, the manufacturing and application of nanotechnological products has reached industrial scale worldwide and has the potential for further growth and expansion. This raises concerns about the unforeseen adverse health effects on both nanoindustry workers and nanoprodut consumers [13, 8].

Zhao L [15] noted that nanoparticles (NPs) released to the work area air might contribute to the cardiopulmonary effects observed in workers. Biomarkers of lung damage, cardiovascular diseases, as well as biomarkers of oxidative stress and inflammation which were associated with the occupational exposure to the studied NPs were found in the workers of the NP factory.

It was experimentally established that the cardiotoxic effect of NPs of titanium oxide, zinc oxide, silver, carbon, silicon dioxide and iron oxide depended on both the toxicity of these compounds and the

size of their nanoparticles [3]. Therefore, the morbidity rate of workers can be due to both the toxicity of the studied substances and the ability of their ultrafine particles to deeply penetrate into the body tissues.

Studies conducted by [12] allowed to obtain results on nanoparticle emissions in real working conditions during various technological processes of manufacturing and use of nanotechnological products. During one of the repeated processes an unexpected and extremely high emission of nanoparticles was registered, which in the long run might endanger the workers' health. Measurements of the actual level of nanoobjects impact have shown the necessity to monitor the work area air and the importance of collective protective measures.

Lebedova J et al. [10] focused their experiment on the acute and subchronic inhalation effects of lead oxide nanoparticles, as in the production environment it is the inhalation exposure that is most likely. The authors found that accumulation of lead oxide NPs in all tissues depended on the duration of exposure and the concentration of PbO NPs, with lungs and kidneys being the most vulnerable among the studied organs. Histological analysis documented numerous morphological changes and tissue damage, mainly in the lungs.

Dumková J [4] had studied the subchronic inhalation effect of lead oxide nanoparticles on mice. Microscopic and ultramicroscopic changes caused by PbO NPs in the primary and secondary target organs (lungs, brain, liver, kidneys, spleen and blood) were specifically determined. Lead content was also found to be the highest in the lungs and kidneys, slightly lower in the liver and spleen; the lowest lead content was found in the brain. Nanoparticles were found in all the studied tissues, with their amount being the highest in lungs and liver. Moreover, in the lungs of animals exposed to PbO NPs the authors found hyperemia, small areas of atelectasis, alveolar emphysema, focal acute catarrhal bronchiolitis, as well as hemostasis with siderophages in some animals. Nanoparticles were located in phagosomes or formed clusters inside cytoplasmic vesicles. Thus, subchronic inhalation exposure of mice to PbO NP causes severe adverse effects at both cellular and tissue levels [4].

The results of Lucie Bláhová's experiment [2] also showed that subchronic inhalation of PbO nanoparticles had caused histopathological changes in mice, mainly found in the lungs and liver and indicating inflammation and a general toxicity reaction.

Thus, both *in vivo* and *in vitro* studies have shown that specific features of NPs surfaces allow them to cross cellular barriers, damage structures and disrupt functions of body cells [1, 9]. However, the mechanisms underlying nanotoxicity have not been fully studied.

Therefore, the social and commercial benefits of nanomaterials should not outweigh the potential adverse effects on human health and the environment associated with occupational and consumer exposure to them, which necessitates comprehensive toxicological studies of nanosized compounds [14].

The current scarcity of data on the toxic effects of nanoscale lead particles on the cardiovascular system makes the study of their cardiovascular effects features relevant.

The purpose of the study was to examine features of morphological changes in the lung, myocardium and aorta under the action of lead sulfide nanoparticles of different sizes in the experimental model of single acute intratracheal administration.

Materials and methods. The experiments were conducted on rats (mean weight of 160–180 g). Animals were kept in the vivarium on a standardized diet with free access to drinking tap water. In simulating intoxication colloidal solutions of lead sulfide (PbS in sodium polyphosphate) with an average size of 26–34 nm (PbS_{26–34nm}) and 50–80 nm (PbS_{50–80nm}), and lead nitrate Pb(NO₃)₂ in ionic form which is well soluble in water were used [7]. The control group was injected with normal saline.

NPs dimensions were determined by electron microscopy. The studied substances were administered intratracheally once at a dose calculated by lead content 5×10^{-3} Mol / L. Toxic effects were assessed 12 days after exposure.

The animals were sacrificed by decapitation under mild ether anesthesia and their internal organs were harvested. All manipulations with animals were performed in accordance with the provisions of the "European Convention for the Protection of Vertebrate Animals, Used for Experimental and Other Scientific Purposes" (Strasbourg, 1985) and approved by the Bioethics Committee of the NAS of Ukraine. The experiment plan is approved by the Bioethics Commission of State Institution "Kundiiev Institute of Occupational Health of the National Academy of Medical Sciences of Ukraine" (Minutes № 5, session of bioethics commission from 23.11.2017).

The heart with aorta and lungs were fixed in 10% neutral formalin, dehydrated in isopropanol and embedded in paraffin (Leica Surgipath Paraplast Regular). Paraffin sections were made on a Thermo Microm HM 360 microtome. The sections were deparaffinized and stained with H&E and azure–eosin.

The slides were studied using Olympus BX51 microscope. Morphometric analysis was performed using software Carl Zeiss (AxioVision SE64 Rel.4.9.1), magnification $\times 200$, $\times 400$.

Aorta wall thickness (mkm), adventitia of aorta thickness (mkm), comparative amount of collagen fibers in tunica adventitia (%), number of elastic membranes in tunica media (conventional units) were examined. The statistical study was performed in Origin Lab version 8.0 using the One-way ANOVA test. Data are presented as medians with smaller and larger quartiles (M[Q1-Q3]), because normality wasn't proven.. The difference was considered statistically significant at $P < 0.05$.

Results of the study and their discussion. After the administration of $PbS_{26-34nm}$ and $PbS_{50-80nm}$ pronounced structural changes in the rats' lungs were found. Dystrophic changes of the epithelial plate, desquamation of dead cells into the bronchial lumen, and accumulation of cellular detritus were found in the bronchial wall. The wall integrity was disrupted at different levels of the small bronchi (Fig. 1). Increased lymphocyte density, emergence of neutrophils and eosinophils were observed around the small and terminal bronchi in the $PbS_{26-34nm}$ and $PbS_{50-80nm}$ group, which is the evidence of inflammatory infiltration. The acinar structure of the respiratory part of the lungs is severely distorted. The relative density of the alveoli at the optical examination was reduced, due to the increased content of the structurally altered stromal elements (paratrachial and paravasal connective tissue) and infiltrated leukocytes. Clusters of macrophages were found in the alveolar lumen, with almost no structurally preserved alveoli present in the $PbS_{50-80nm}$ group, the reorganization of the acinar structure of the lungs has been severe until the complete loss of the respiratory part of the lungs morphology. Most large and medium caliber blood vessels were structurally preserved, dilated, with the stasis of blood cells observed. Administration of $Pb(NO_3)_2$ to rats also caused a pronounced inflammatory process in the lungs with the development of degenerative changes in the bronchial wall and in the respiratory part of the lungs. Changes in the respiratory part of the lungs were similar to the disorders in the $PbS_{26-34nm}$ and $PbS_{50-80nm}$ groups, but relatively preserved areas of the lungs were still found (individual alveoli, blood capillaries). Results of histological examinations allowed to state the development of an inflammatory reaction in the respiratory part of the lungs with impaired morphology of the pulmonary acinuses and dystrophic changes of the bronchial mucosa after administration of $PbS_{26-34nm}$, $PbS_{50-80nm}$ and $Pb(NO_3)_2$.

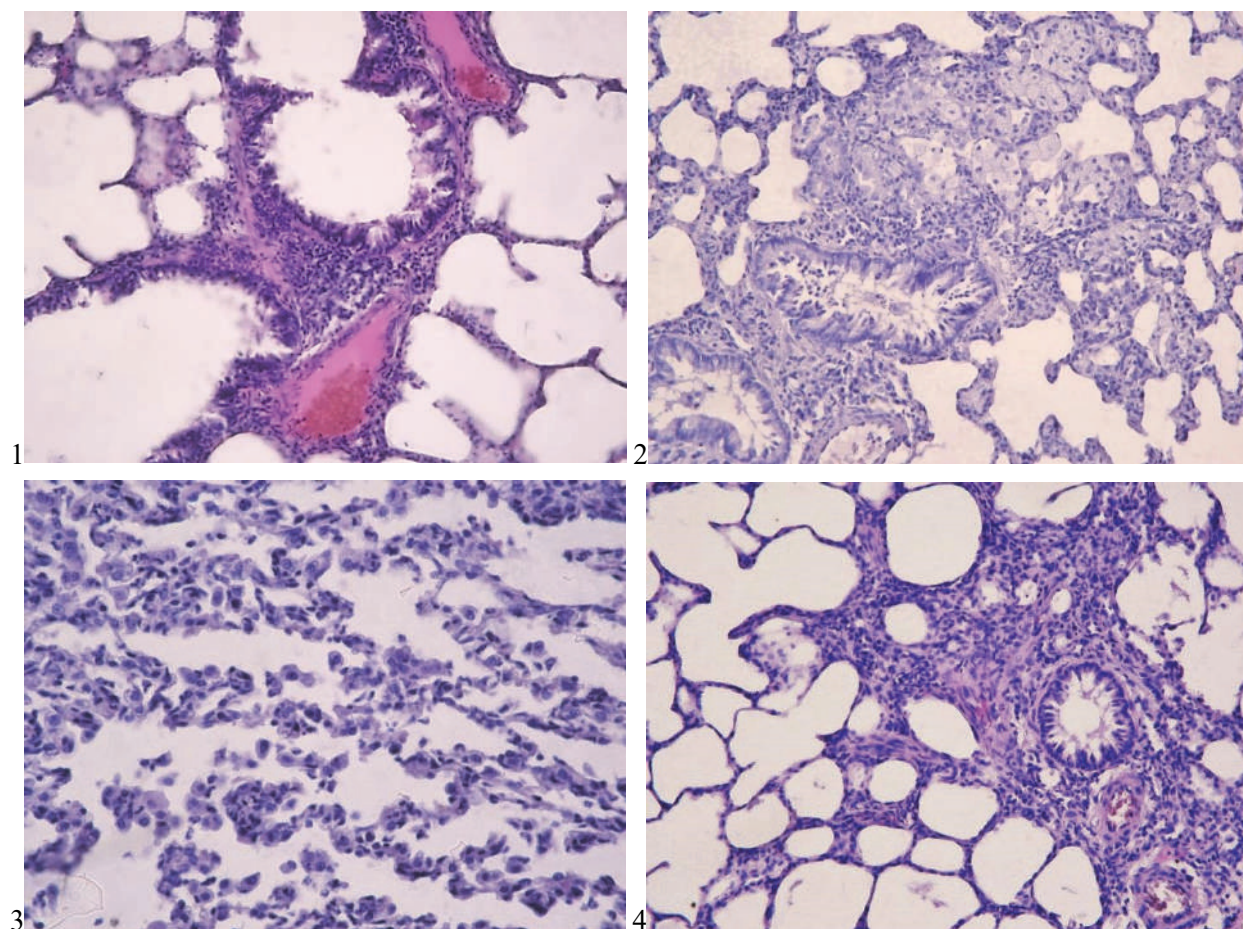


Fig. 1. Rats' lungs after intratracheal administration of lead. Accumulation of macrophages and damaged epitheliocytes in the lumen of the alveoli and bronchi in the $PbS_{26-34nm}$ and $PbS_{50-80nm}$ groups. Note: 1 – control; 2 – $PbS_{26-34nm}$; 3 – $PbS_{50-80nm}$; 4 – $Pb(NO_3)_2$ H&E, ob. 20, e.p. 10.

In all samples the general morphology of the rats' heart was preserved, endo-, myo- and epicardium were detected as in the control group. But in the myocardium structural changes were observed. A nonspecific disorder found in all experimental groups was the increased interstitial space, which in the PbS_{26-34nm} and PbS_{50-80nm} groups was more pronounced in the atria.

In the PbS_{26-34nm} group nuclear diameters of atrial cardiomyocytes were increased with hypochromic staining of cytoplasm and optically transparent areas around the nuclei, which is the evidence of their dystrophic changes. In the PbS_{50-80nm} group, on the contrary, the diameters of cardiomyocytes and nuclei were reduced, and the transverse striation of cardiomyocytes distorted. In the group with Pb(NO₃)₂ administration structural changes in the myocardium were weakly expressed (slightly increased interstitial space), with the transverse striation partially preserved (fig. 2).

No significant disorders at the level of the endocardium and epicardium in the comparison groups (PbS_{26-34nm}, PbS_{50-80nm}, Pb(NO₃)₂) were detected. Figure 3 shows the results of morphometric evaluation of the cardiomyocytes diameter of the comparison groups. Morphometric changes allow us to state the damage to the ventricular myocardium in the PbS_{26-34nm} and PbS_{50-80nm} groups represented by the reduced diameter of cardiomyocytes; in the atrium, on the other hand, edema and dystrophic changes were observed in the PbS_{26-34nm} group, and in the PbS_{50-80nm} group – a decreased diameter (similar to changes in the ventricle). No changes of the kind were detected in the Pb(NO₃)₂ group.

The analysis of the results of histological and morphometric studies of the aortic wall did not reveal any significant morphofunctional or dystrophic changes. The aortic wall in the experimental groups was structurally unchanged, the layers of the vessel were clearly differentiated (fig. 4). The main wall thickness was represented by the *t.media*, comprising 8 to 12 elastic membranes. The number and density of elastic membranes did not differ between the comparison groups (Fig. 3). *T.adventicia* made up almost one third of the aortic wall. There is a dense network of collagen fibers and single blood vessels in the connective tissue of the outer tunica.

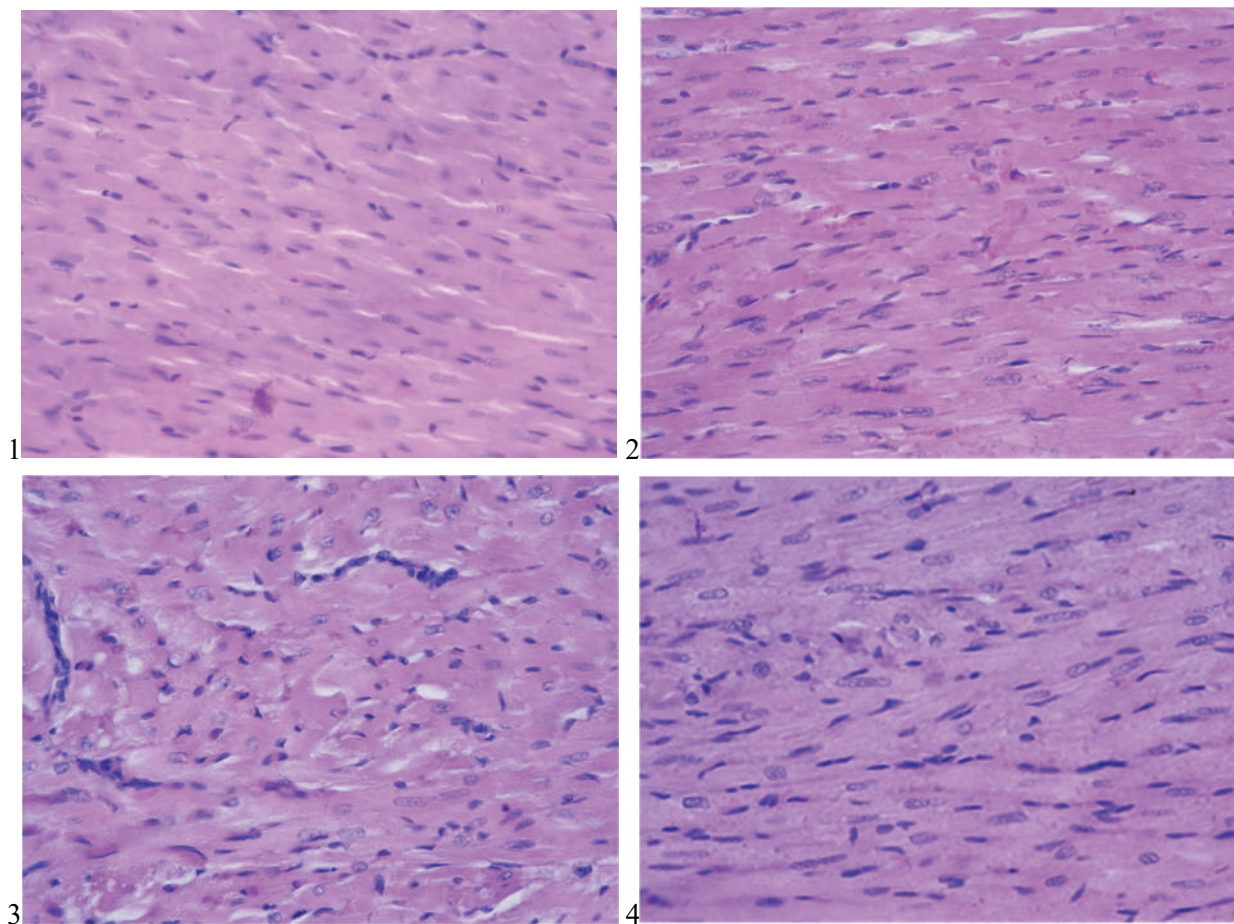


Fig. 2. Ventricular myocardium of rats after intratracheal administration of lead. Note: 1 – control; 2 – PbS_{26-34nm}; 3 – PbS_{50-80nm}; 4 – Pb(NO₃)₂. H&E, ob. 40, e.p. 10.

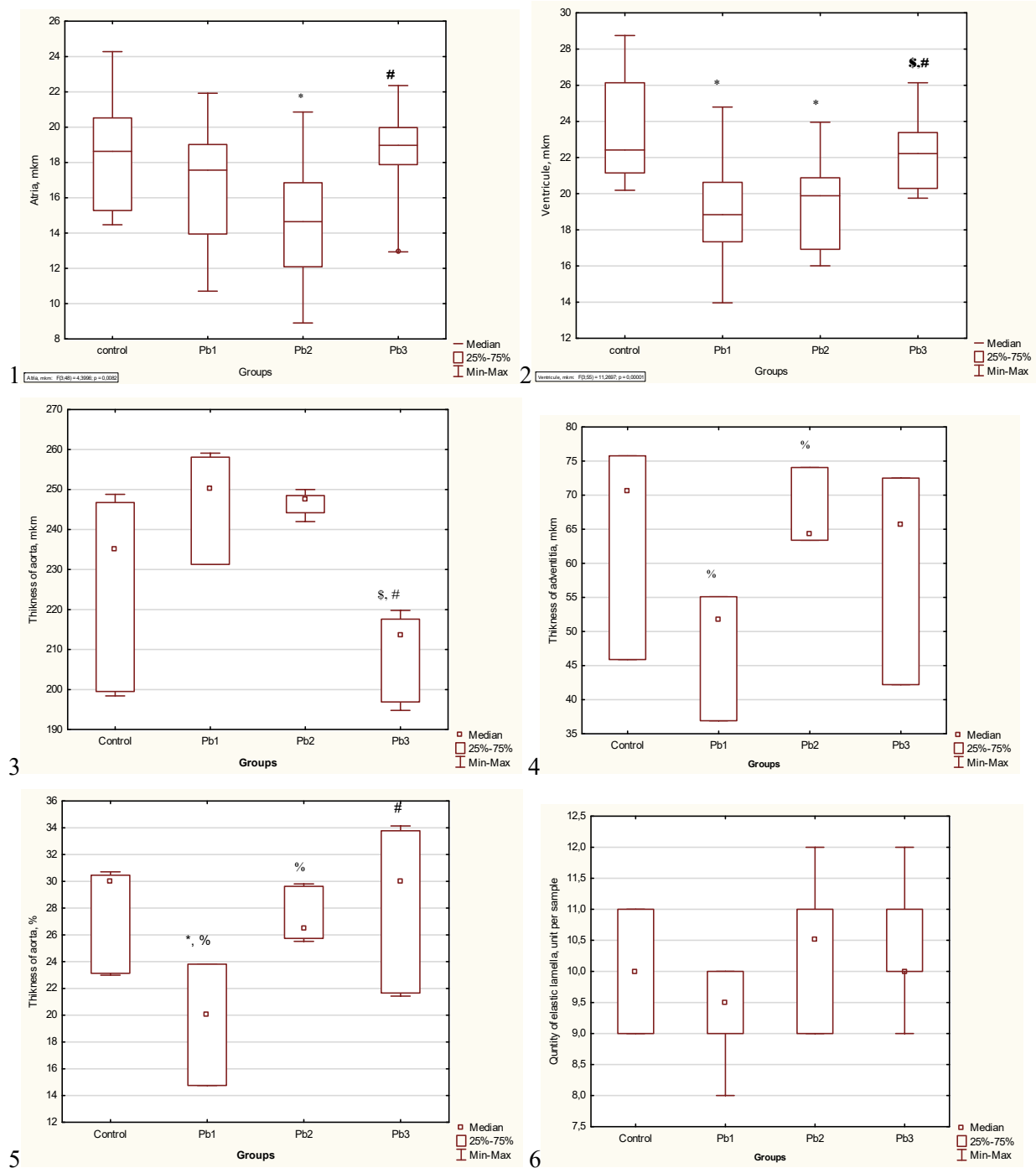


Fig. 3. The diameter of the rats' heart cardiomyocytes and aorta after intratracheal administration of lead. Note: 1 – atrium; 2 – a ventricle; 3 – aortic thickness, μm ; 4 – thickness of the adventitia, μm ; 5 – relative thickness of the adventitia, %; 6 – the number of elastic membranes in the aortic wall, units per sample. * – statistically significant difference from control ($P < 0,05$); \$ – statistically significant difference from PbS_{26–34nm} ($P < 0,05$), # – statistically significant difference from PbS_{50–80nm} ($P < 0,05$), % – statistically significant difference between Pb ($P < 0,05$) NP.

In our previous work [6] we studied the cardiovasotoxic effect of PbS NP 26–34 nm and 50–80 nm under subchronic intraperitoneal uptake. The results of the conducted experiments indicated damage to the rats' heart myocardium, which consisted in the increased interstitial space between the cardiomyocytes fibers, cardiomyocyte dystrophy, and blood stasis in the ventricular microvessels. Greater sensitivity of the atrial myocardium to the toxic effects of Pb NP was found. Structural changes in the aorta included dissection of the elastic membranes of the aortal *m. tunica*, decreased density of the adventitial membrane connective tissue and showed a tendency to progressive changes.

In this experiment we aimed at studying the effect of NP PbS 26–34 nm and 50–80 nm on the heart and aorta in acute intratracheal uptake, as it is the inhalation exposure that is most likely in the production environment.

It turned out that the heart myocardium had also experienced the toxic effects of PbS NP of different sizes, which affected the morphological changes of cardiomyocytes. These changes were mild

and to a greater extent emerged in the atrium. The general morphological organization of the heart chambers remained intact and the heart tunics were clearly seen. We concluded that there was a mild toxic effect of PbS NP on the myocardium under a single intratracheal administration, and morphological changes, such as increased interstitial space between cardiomyocytes, dystrophic changes in their cytoplasm did not have any obvious differences between PbS NP of different sizes. At the light-optical level there were no changes in the aortic wall.

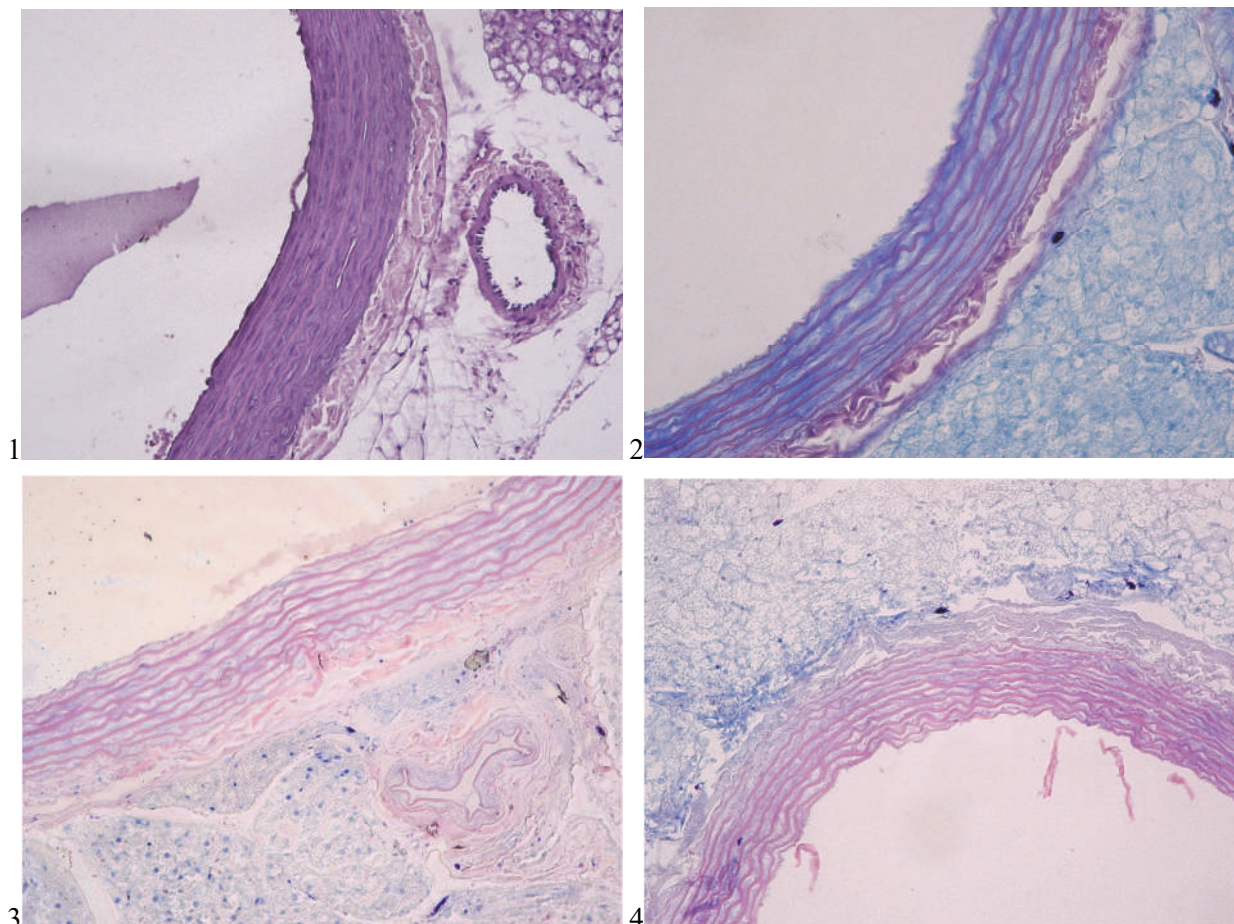


Fig. 4. The rats' aorta after intratracheal administration of lead. Note: 1 – control; 2 – PbS_{26-34nm}; 3 – PbS_{50-80nm}; 4 – Pb(NO₃)₂. H&E (1), azure-eosin (2,3,4). Ob. 20, e.p. 10.

Li Q. has experimentally established that the inhalation effect of PbS NP caused inhibition of the activity of SOD, T-AOC and an increase in the content of MDA in blood serum; both effects were also observed in the lung tissue of rats and were accompanied by a pronounced inflammatory reaction. Therefore, the main mechanism of pulmonary toxicity of PbS NP may be oxidative stress and inflammatory response in lung tissue [11].

According to other authors [4], pathological changes at both cellular and tissue levels during inhalation exposure of mice to PbO NP do not correspond to the concentration of lead in this organ, but to the number of nanoparticles detected in the cells.

In the study of molecular and cellular mechanisms promoting the elimination of nanoparticles variability between target organs was recorded. In particular, it was found that the elimination of ionic lead and PbO NP from the lungs and liver was effective, with lead almost completely eliminated from the lungs, which helped to restore the physiological state of lung tissue [5].

In our experiment we have recorded morphological changes of different intensity of organs resulting from acute intratracheal exposure to PbS NPs of different sizes and Pb(NO₃)₂: toxicants had a more pronounced damaging effect on the lungs and myocardium; the most resistant to their influence were the aortic wall tissues. We have not found any morphometrically significant relationship between the size of the studied lead nanoparticles and the severity of their cardiovasotoxic effects, although the histological picture of toxic manifestations is more expressed in the group of NP PbS_{50-80nm}.

Conclusion

With a single intratracheal administration of lead cardioasototoxic effect of PbS_{26-34 nm} and PbS_{50-80nm} NP was manifested more compared with a significantly milder effect caused by the action of the ionic form of lead – Pb(NO₃)₂.

The cardiotoxic effect of lead nanoparticles mainly affected the atrial myocardial cardiomyocytes, and the aortic wall remained almost unaffected. The most pronounced structural changes were observed in the lungs, which may be due to the route of a toxicant administration.

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Реферати

**ОРГАНОТОКСИЧНИЙ ЕФЕКТ
ОДНОКРАТНОГО ІНТРАТРАХЕАЛЬНОГО
ВВЕДЕННЯ НАНОЧАСТИНОК СВИНЦЮ
РІЗНОГО РОЗМІРУ**

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В експерименті вивчався кардіовазотоксичний ефект одноразового інтратрахеального введення наночастинок свинцю різного розміру. Використовували колоїдні розчини сульфиду свинцю (PbS у поліфосфаті натрію) з середнім розміром 26–34 нм та 50–80 нм, а також нітрат свинцю Pb(NO₃)₂ в іонній формі, який добре розчинний у воді. Токсичні ефекти оцінювали через 12 днів після впливу. Морфологічні зміни були виявлені в міокарді (більшою мірою в передсердях). Після введення наночастинок PbS_{26-34nm} та PbS_{50-80nm} визначались незначні порушення, такі як збільшений інтерстиціальний простір та дистрофічні зміни окремих кардіоміоцитів. Таким чином, при інтратрахеальному введенні свинцю токсичний ефект наночастинок PbS_{26-34nm} та PbS_{50-80nm} виявлявся більшою мірою порівняно з ефектом, спричиненим дією іонної

**ОРГАНОТОКСИЧЕСКИЙ ЭФФЕКТ
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РАЗНОГО РАЗМЕРА**

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В эксперименте изучался кардиовазотоксический эффект однократного интратрахеального введения наночастиц свинца разного размера. Использовали коллоидные растворы сульфида свинца (PbS в полифосфате натрия) со средним размером 26–34 нм и 50–80 нм, а также нитрат свинца Pb(NO₃)₂ в ионной форме, хорошо растворимый в воде. Токсические эффекты оценивали через 12 дней после воздействия. Морфологические изменения были обнаружены в миокарде (в большей степени в предсердиях). После введения наночастиц PbS_{26-34nm} и PbS_{50-80nm} определялись незначительные нарушения, такие как увеличенное интерстициальное пространство и дистрофические изменения отдельных кардиомиоцитов. Таким образом, при интратрахеальном введении свинца токсический эффект наночастиц PbS_{26-34nm} и PbS_{50-80nm} был выявлен в большей степени по сравнению с эффектом, вызванным действием

форми свинцю $Pb(NO_3)_2$. Токсичний ефект наночастинок свинцю спостерігався в основному в кардіоміоцитах міокарда передсердь, в той час як стінка аорти залишалася майже інтактною. Найбільш виражені структурні зміни спостерігалися в легенях та бронхах, що може бути обумовлено способом введення токсикантів.

Ключові слова: свинець, наночастинки, інтратрахеальна інтоксикація, морфологічні зміни, міокард, аорта, легені.

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ионной формы свинца $Pb(NO_3)_2$. Токсический эффект наночастиц свинца наблюдался в основном в кардиомиоцитах миокарда предсердий, в то время как стенка аорты оставалась почти интактной. Наиболее выраженные структурные изменения наблюдались в легких и бронхах, что может быть обусловлено способом введения токсикантов.

Ключевые слова: свинец, наночастицы, интратрахеальная интоксикация, морфологические изменения, миокард, аорта, легкие.

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ULTRASTRUCTURAL REMODELING OF RAT SUBMANDIBULAR GLANDS IN CHRONIC ETHANOL INTOXICATION

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The study presents a detailed analysis of the ultramicroscopic structure of the rat submandibular glands in chronic ethanol intoxication at the later stages of the experiment. It has been established that prolonged exposure to ethanol at the later stages of the experiment was characterized by the appearance of signs of adaptive-compensatory responses of the parenchymal elements and vessels of the blood microvascular system with the emergence of dystrophic changes and apoptosis of ductal epithelium; however, no complete regeneration of the structure was detected.

Keywords: ethanol, salivary glands, acini, ducts, rats.

The work is a fragment of the research project "Experimental morphological study of the effect of cryopreserved cord blood products and embriofetoplacental complex (EFPC), diphereline, ethanol and 1 % methacrylate on the morphofunctional condition of several internal organs", state registration No. 0119U102925.

Chronic ethanol intoxication causes multiple alterations in the structure and functions of the oral cavity organs; however, the study was focused on the prevalence and intensity of diseases of the oral mucosa and periodontal diseases. The findings have shown that alcohol consumption is accompanied by salivary dysfunction, as well as the destructive changes in the salivary glands; however, these data are often fragmentary and sometimes ambiguous [6]. Ethyl alcohol, due to its physicochemical properties, is able to easily penetrate through the cell membranes and have both direct and indirect toxic effects, which leads to the matrix and transport dysfunction of membranes and the formation of adaptive changes in long-term effect of ethanol, manifested by elevated cholesterol, thickening of the phospholipid layer and higher membrane density, which in turn is accompanied by alterations in the mode of functioning of enzyme, receptor and immune complexes [2].

Electron microscopic method, used to investigate ultrastructural changes in tissues, has established that at the early stages of the experiment, chronic ethanol intoxication causes significant changes in both parenchymal elements and vessels of the blood microvascular system, which is expressed by intensification of secretion in the acini and increased functional activity of the ductal system; therefore, the study on the effect of ethanol at the late stages enables disclosure of a full picture of restructuring of the submandibular glands during the experiment.

The purpose of the work was to study structural changes in the elements of the rat submandibular glands' lobules in chronic ethanol intoxication at late stages of the experiment.

Materials and methods. 20 outbred albino rats were involved into electron microscopic study. The rodents were administered with 12 mg/kg 40° ethanol 4 times a day directly into the stomach [4]. The animals were killed under 25 mg/kg thiopental anesthesia overdose on day 12 and 30 of the experiment. The fragments of submandibular glands were embedded into epon-812 according to standard procedure [1].

Ultrathin sections were made on the LKB-3 (Sweden) ultramicrotome. Contrast staining of the sections were performed first with 1% uranyl acetate solution in methanol, and then with lead citrate according to Reynolds [1].

The sections were studied in the PEM-125K electron microscope (serial number 38-76, TU 25-07-871-70) at accelerating voltage (50 - 75) kW.

Animal housing and experiments on them have been carried out in compliance with the “General Ethic Rules for Conducting Experiments on Animals”, adopted by the I National Congress on Bioethics and the requirements of international principles of the “European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes” [7].

Results of the study and their discussion. On day 12 of the experimental model of chronic ethanol intoxication in the cells of the acini an eccentric position and compaction of nuclei of irregular shape was noted and increase in the amount of heterochromatin was observed. The number of secretory granules in the cytoplasm decreased. They were of different diameters, different densities of contents, oblong and conjoined in places. In some epitheliocytes of the acini there were electron transparent vacuoles, which were located near the nucleus. In the intercellular spaces, cytoplasmic evaginations on the lateral surfaces and extended fissures near the basal surface were detected (fig. 1a).

Venules with signs of edema of the vascular wall were found in the periductal interstitium. Endotheliocytes had elongated shape. The lumen of the vessels was completely filled with blood corpuscles, as evidenced by previously obtained data from the morphometric study, where on day 12 the thickness of the vascular wall significantly increased by 20.1% compared with day 9 of the experiment and was by 6.3% greater compared to the value in controls ($p < 0.05$) [3]. Interstitial tissue was represented by amorphous substance and collagen fibers (fig. 1b).

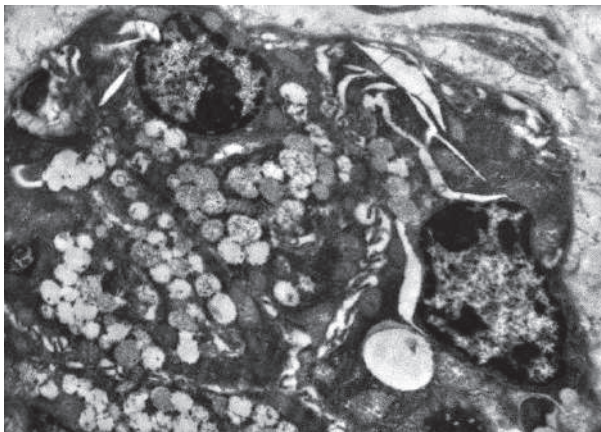


Fig. 1a. Extension of fissures in the seromucous cells of the acini of rat submandibular glands on day 12 of the experiment. Electronogram. 6000×magnification.

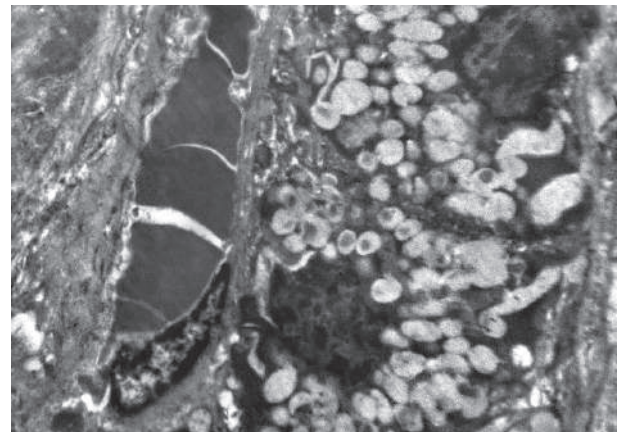


Fig. 1b. Venule in the interstitial tissue on day 12 of the experimental model of chronic ethanol intoxication. Electronogram. 7000×magnification.

The cells of the striated ducts had a prismatic shape. The nuclei were enlarged, oblong with a predominance of euchromatin. A decrease in the width of the folds of the basal striation, a decrease in the number of mitochondria was noted. The basement membrane was smooth. In the periductal interstitium, amorphous substance predominated over the collagen fibers, and capillaries with endothelial cells of elongated irregular shape were found. The cytoplasm of endothelial cells was filled with different electron density contents and transport vesicles. In the lumen of the capillaries erythrocytes were oblong (fig. 2a).

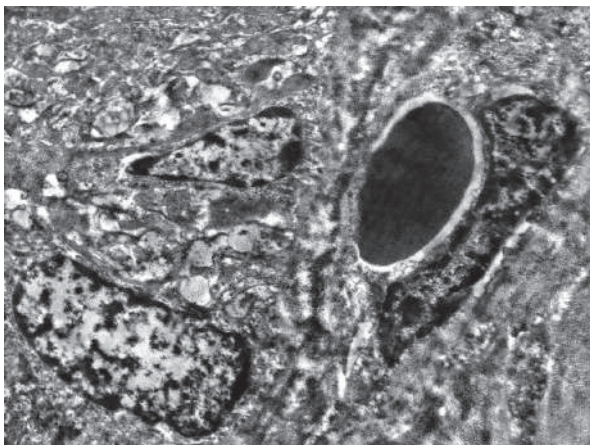


Fig. 2a. Capillary in the periductal interstitium of submandibular glands in rats of the experimental group on day 12 of the experiment. Electronogram. 8000×magnification.

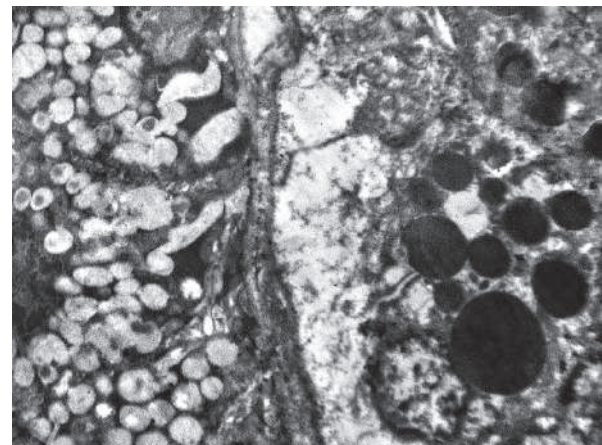


Fig. 2b. Detachment of plasmalemma of the granular ducts of rat submandibular glands on day 30 of the experiment. Electronogram. 7000×magnification.

On day 30 of the experiment in the cells of the acini, the nucleus moved to the center of the cells and numerous electron-light granules of various shapes were detected. Dark electron-dense gaps of the cytoplasm were found on the basal surfaces. Granular ducts in rats are responsible for maintaining and providing a system of local mechanisms for regulating vascular blood supply. Their epitheliocytes had basally located nuclei with a pronounced polymorphism of the granules, which were different in size and mostly electron-dense. Large fissures of various shapes were detected between the plasma membrane and the basement membrane (fig. 2b). In some cells of the ductal epithelium, heterogeneity of the cytoplasm with electron-light vacuole-like structures was noted; the nuclei were of various shapes, hyperchromic, and cells with crescent-shaped nuclei were detected, the central part of which was filled with transparent content (fig. 3a). Elongated fissures were noted in the cells of the striated ducts at the border with the basement membrane. Fibroblasts and adjacent bundles of collagen fibers were detected among the amorphous substance in the surrounding periacinar interstitium. Small lymphocytes and macrophages were noted (fig. 3b).

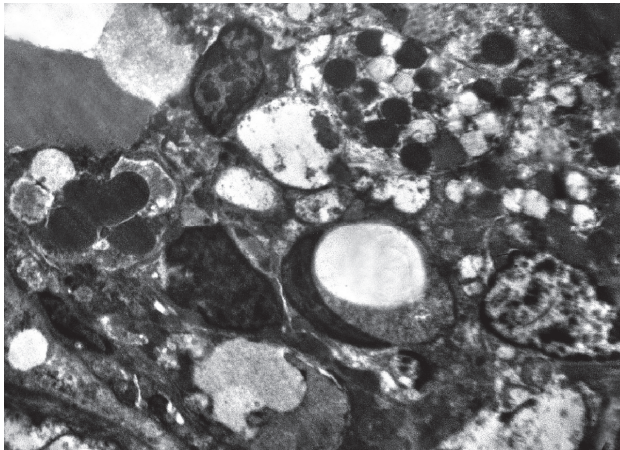


Fig. 3a. The apoptosis phenomena in granular duct cells of rat submandibular glands on day 30 of the experiment. Electronogram. 7000×magnification.

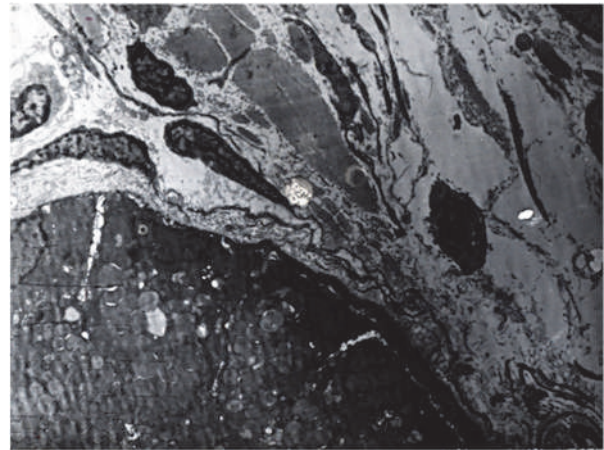


Fig. 3b. Striated duct and interstitial periductal tissue on day 30 of the experiment. Electronogram. 6000×magnification.

Thus, on day 12 of ethanol effect, the signs of decreased secretion by seromucocytes of the acini was detected, which was confirmed on the electronograms by compaction of nuclei, a decrease in the number of secretory granules and the appearance of cytoplasmic projections on the intercellular surfaces. The cells of the striated ducts took on a typical prismatic shape, a decrease in the number of mitochondria and folds of the basal labyrinth indicated a decrease in the saliva modification processes by epitheliocytes. Due to the decrease in hydration of the interstitium, contributed by hypersalivation, at the early stages of the experiment, the vessels of the blood microvascular system of the interstitial connective tissue increased in volume and blood corpuscles were visualized.

On day 30 of the experimental model of chronic ethanol intoxication the formation of adaptive-compensatory changes of parenchymal elements of the submandibular glands in response to the long-term effect of ethanol. In the seromucous cells of the acini, compaction of the basal parts of the cytoplasm was detected, expressed by dark areas on the electrogram. The nuclei moved to the center and were compacted. The granules were polymorphic, sometimes rod-shaped, electron-light, and conjoined in some areas. The above changes in the acini indicated a compensatory adjustment after hypersalivation at the early stages of the experiment and restructuring of the secretory apparatus mainly in the direction of carbohydrate synthesis. The epitheliocytes of the striated ducts had numerous folds of basal striation oriented perpendicular to the basal surface of the cells, were narrow and high with numerous vacuole-like structures in the cytoplasm. Clear catenate fissures were visible in the intercellular spaces. The presence of large electron-dense granules and the presence of optically bright areas in the basal parts starting from the basement membrane to almost a third of the cells were noted in the granular ducts. Heterogeneity of the cytoplasm and the emergence of the nuclei of the irregular shape indicate the occurrence of apoptosis. The established changes in the ductal epithelium indicate partial depletion of cells with the appearance of signs of dystrophy, and the full use of their adaptation to support salivation processes during prolonged exposure to ethanol. The increased number of macrophages and lymphocytes in the periductal connective tissue was detected, indicated about their active role in providing a local protective barrier.

In the previous experiments on the rat submandibular glands exposed to the effect of other stimuli, namely 1% methacrylate, parenchymal components showed signs of inflammation and

decompensation of the microcirculation, which led to significant vasodilation. Enlargement of the diameters of the duct lumen was observed at the final stages of the experiment. The number of plasma cells in the peri-acinar and periductal epithelium increased significantly. There was no tendency to recover [5], which obviously proves a more toxic effect on the parenchymal elements of the stimulus, to which the salivary glands respond by inflammation with dysfunction, while the effect of ethanol, as a metabolite, causes less reactive outcomes with the emergence of dystrophic changes and apoptotic phenomena without inflammatory process. The observations indicate that the reaction of the structural components of the submandibular glands depends on both the modality of the active stimulus and its chemical affiliation.

Conclusion

Chronic ethanol intoxication at the later stages of the experiment is characterized by the appearance of signs of adaptive-compensatory reactions of parenchymal elements and vessels of the blood microvascular system with the emergence of dystrophic changes and apoptosis of ductal epithelium caused by prolonged effect of ethanol and its products. However, no complete regeneration of the structure was detected.

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Реферат

УЛЬТРАСТРУКТУРНЕ РЕМОДЕЛЮВАННЯ ПІДНИЖНЬОЩЕЛПНИХ СЛИННИХ ЗАЛОЗ ЩУРІВ ПРИ ХРОНІЧНІЙ ІНТОКСИКАЦІЇ ЕТАНОЛОМ

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В даному дослідженні було проведено детальне вивчення ультрамікроскопічної будови піднижньощелепних слинних залоз щурів за хронічної інтоксикації етанолом на пізніх термінах експерименту та було встановлено, що тривала дія етанолу на пізніх стадіях експерименту характеризується появою ознак адаптивно-компенсаторних реакцій паренхіматозних елементів та судин гемомікроциркуляторного русла з виникненням дистрофічних змін та явищами апоптозу протокового епітелію, однак повного відновлення структури не відбувалось.

Ключові слова: етанол, слинні залози, кінцеві відділи, протоки, щури.

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УЛЬТРАСТРУКТУРНОЕ РЕМОДЕЛИРОВАНИЕ ПОДНИЖНЕЧЕЛЮСТНЫХ СЛЮННЫХ ЖЕЛЕЗ КРЫС ПРИ ХРОНИЧЕСКОЙ ИНТОКСИКАЦИИ ЭТАНОЛОМ

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В данном исследовании было проведено детальное изучение ультрамикроскопического строения поднижнечелюстных слюнных желез крыс при хронической интоксикации этанолом на поздних терминах эксперимента. Было установлено, что длительное воздействие этанола на поздних стадиях эксперимента характеризуется появлением признаков адаптивно-компенсаторных реакций паренхиматозных компонентов и сосудов гемомикроциркуляторного русла с возникновением дистрофических изменений и явлениями апоптоза протокового эпителия, однако полного восстановления структуры не происходило.

Ключевые слова: этанол, слюнные железы, концевые отделы, протоки, крысы.

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MORPHOMETRIC ANALYSIS OF MAXILLARY TUBERCLES IN CHILDREN TO DETERMINE THE POSSIBILITY OF THEIR USE AS A SUPPORT FOR DISTALIZATION

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High intensity and prevalence of carious process lead to premature extraction of deciduous teeth and the creation of favorable conditions for development of anomalies and deformities. To improve the efficacy in treatment of dentoalveolar malocclusions which occur as a result of pathological mesial displacement of the maxillary lateral teeth, we considered the possibility of using the maxillary tubercles as a self-sustained or additional distal support to move the permanent molar in the distal direction. As a result of studying the features of the maxillary tubercles structure, we proposed their distribution according to their form: a) flat; b) sloping; c) convex; by height into: a) low (up to 2 mm); b) medium (2-3 mm); c) high (more than 3 mm); by length into: a) short (up to 5 mm); b) medium (5-8 mm); c) long (more than 8 mm). The prevalence of maxillary tubercles different in sizes and shapes in children of different age groups was studied, as well as the orthodontic apparatus for molars distalization using a high convex maxillary tubercle as a distal support.

Key words: children, maxillary tubercles, pathological mesial displacement of the first permanent molars, anchor system, molars distalization.

The work is a fragment of the research project "Clinical and experimental justification for improving the quality of orthopedic treatment in patients with dentoalveolar pathology", state registration No. 0120U000573.

Due to the high intensity of caries and premature removal of deciduous teeth, especially molars, there are favorable conditions for the development of anomalies and deformations in the sagittal, transverse and vertical planes [1, 2, 8, 9]. Mesial migration of lateral teeth is especially pronounced, which in case of premature extraction of deciduous teeth and untimely prosthetics contributes to the occurrence of dental anomalies, and the degree of displacement depends on the time of loss of deciduous molars [3, 4].

A necessary and important stage of orthodontic treatment is to correct the position of displaced permanent molars, especially in the upper jaw. Therefore, for their bodily distal movement often use fixed orthodontic appliances, one of which components is the Nance basis, which is located on the palate and acts as a mesial support in the distalization of molars [5, 6, 7, 12, 14]. However, the problem of the fixing "anchor" loss, protraction of the dentition frontal part, combination of different anchor systems during the orthodontic treatment remains relevant [10, 11, 13, 15].

Therefore, given the causes and consequences of premature milk molars extraction, there is a need for early diagnosis of pathological mesial teeth transfer, development of measures aimed at improving methods for prevention and treatment of this pathology.

The purpose of the study was to perform morphometric analysis of maxillary tubercles in children to determine the possibility of their use as a distal support in the distalization of molars.

Materials and methods. To study the structure of the maxillary tubercles, we examined 60 children without dental anomalies (group I), which were divided into the following subgroups: 7-8 years (IA), 9-10 years (IB) and 11-12 years (IC) (20 children in each group) and studied the shape and size of 120 maxillary tubercles. During the examination, they used the "Map of determining the condition of maxillary tubercles", which contained the passport data of the child, year of birth, dental formula, data on the shape and size of the maxillary tubercles. Each child underwent the maxillary contouring with alginate masses, and using the obtained diagnostic models the shape of the maxillary tubercles was determined and their size was measured using a caliper and orthodontic dental gauge.

To determine the length of the maxillary tubercle, the orthodontic gauge was placed on the tubercle slope and the distance was measured from the distal contact surface of the last molar to the bottom of the maxillary pterygoid notch, which is formed by the lower fibers of the internal pterygoid muscle fixed to the alveolar tubercles and posterolateral surface of the palatal bone's horizontal part.

When determining the height of the maxillary tubercle, the depth gauge of the caliper was placed transversely to the bottom of the maxillary pterygoid notch so that the rod could touch the highest point of the tubercle. After fixing the position of the caliper, the height of the maxillary tubercle was determined (distance from the bottom of the maxillary pterygoid notch to the highest point on the maxillary alveolar tubercle).

For statistical processing of the study results we used the generally accepted Student-Fisher method, variational-statistical method of analysis on a personal computer using a package of statistical software "Microsoft Excel – 2007" and "Statistica-6.0".

Statistical processing of the study results was performed by calculating the following indices: arithmetic mean (M), arithmetic mean error (m), the reliability of the differences in the results (P), which were considered significant with a probability factor less than 0.05.

To perform distal movement of permanent molars using the maxillary tubercle as a distal support, we have proposed an orthodontic appliance for distalization, which permits to use high and convex maxillary tubercles as a self-sustained or additional anchor system.

Results of the study and their discussion. In cases when in premature extraction of deciduous molars prophylactic prosthetics was not performed and pathological mesial displacement of the first permanent molar was revealed, we considered the possibility of using the maxillary tubercle as a self-sustained or additional intraoral distal support to move the permanent molar, which prompted us to study in detail topographic and anatomic features of maxillary tubercles structure in children.

Thus, in order to improve the methods of molar distalization, we studied the structural features of 120 maxillary tubercles in children.

As our studies have shown, maxillary tubercles differ in shape and size. When studying the tubercles' size, we paid attention to their height and length, and as a result of our studies, we proposed the following distribution of maxillary tubercles.

1. By shape: a) flat; b) sloping; c) convex.
2. In height: a) low (up to 2 mm); b) medium (2-3 mm); c) high (more than 3 mm).
3. In length: a) short (up to 5 mm); b) medium (5-8 mm); c) long (more than 8 mm).



Fig. 1. Flat maxillary tubercle.



Fig. 2. Sloping maxillary tubercle.



Fig. 3. Convex maxillary tubercle.

Varieties of the shape of the humps of the upper jaw are shown in Fig.1, 2, 3.

The prevalence of different shape maxillary tubercles in different children's subgroups is presented in table 1.

From the data presented in the table it is seen that in the subgroup IA the prevalence of flat tubercles was (22.5 ± 6.6) %, significantly higher ($p < 0.05$) was the prevalence of sloping tubercles, which was (50.0 ± 7.9) %, and convex ones - (27.5 ± 7.1) %. In subgroup IB, the prevalence of flat tubercles was (22.5 ± 6.6) %, sloping - (42.5 ± 7.8) % and convex - (35.0 ± 7.5) %, with a significant difference between the prevalence of flat, sloping and convex tubercles not being detected. In subgroup IC, the prevalence of flat tubercles was (20.0 ± 6.3) %, sloping tubercles - (32.5 ± 7.4) % and convex ones - (47.5 ± 7.9) % ($p < 0.05$).

Thus, at the age of 7 to 12 years, there is a change in the maxillary tubercles shape from sloping to convex one, due to the growth of the alveolar process and the preparation of the body to eruption of other permanent molars.

Table 1

Distribution of maxillary tubercles by shape in children during the mixed occlusion period

Shape of tubercle	Age of children (years)					
	7-8 (IA)		9-10 (IB)		11-12 (IC)	
	n	%	n	%	n	%
Flat tubercle	9	22.5±6.6	9	22.5±6.6	8	20.0±6.3
Sloping tubercle	20	50.0±7.9*	17	42.5±7.8	13	32.5±7.4
Convex tubercle	11	27.5±7.1	14	35.0±7.5	19	47.5±7.9*

Note: * - $p < 0.05$, compared to the prevalence of flat tubercles.

The dynamics of changes in the height of the maxillary tubercles in children during the mixed occlusion period are shown in table 2.

Table 2

Distribution of maxillary tubercles by height in children during the mixed occlusion period

Tubercle's height	Age of children (years)					
	7-8 (IA)		9-10 (IB)		11-12 (IC)	
	n	%	n	%	n	%
low	12	30.0±7.3	7	17.5±6.0	5	12.5±5.2
medium	17	42.5±7.8	19	47.5±7.9*	16	40.0±7.8*
high	11	27.5±7.1	14	35.0±7.5	19	47.5±7.9*

Note: * - $p < 0.05$, compared to the prevalence of low tubercles.

Analysis of the obtained data shows that the height of maxillary tubercles in children during the mixed occlusion period changes. Thus, in children of subgroup IA the prevalence of low tubercles was (30.0±7.3) %, medium - (42.5±7.8) %, high - (27.5±7.1) % and a significant difference between these indices was not detected. In subgroup IB, the prevalence of low tubercles was (17.5±6.0) %, and the prevalence of medium maxillary tubercles reliably ($p < 0.05$) increased, making (47.5±7.9) %, and the percentage of high tubercles was (35.0±7.5) %. In subgroup IC it was found that the prevalence of low tubercles was (12.5±5.2) % and significantly higher ($p < 0.05$) were the prevalence of medium and high maxillary tubercles, which were, respectively, (40.0±7.8) % and (47.5±7.9) %.

Thus, during the mixed occlusion period, the height of the maxillary tubercles gradually changes from low to medium in 7-9 years and from medium to high in 10-12 years, depending on the growth and development of the jaw and the alveolar process, the stage of permanent molars eruption and, possibly, genetically determined features of child development.

The dynamics of changes in the length of the maxillary tubercles in children during the mixed occlusion period are shown in table 3.

Table 3

Distribution of maxillary tubercles by length in children during the mixed occlusion period

Tubercle's length	Age of children (years)					
	7-8 (IA)		9-10 (IB)		11-12 (IC)	
	n	%	n	%	n	%
short	9	22.5±6.6	6	15.0±5.7	10	25.0±6.9
medium	19	47.5±7.9*	21	52.5±7.9*	13	32.5±7.4
long	12	30.0±7.3	13	32.5±7.4	17	42.5±7.8

Note: * - $p < 0.05$, compared to the prevalence of short tubercles.

The data in table 3 indicate that in children aged 7-8 years (IA) the prevalence of middle maxillary tubercles (47.5±7.9) % was significantly higher ($p < 0.05$), the prevalence of short maxillary tubercles was (22.5±6.6) %, and long ones - (30.0±7.3) %. In children aged 9-10 years (IB) (15.0±5.7) % of short, (52.5±7.9) % ($p < 0.05$) of medium and (32.5±7.4) % of long maxillary tubercles were revealed. At the age of 11-12 years (IV), the prevalence of short, medium and long maxillary tubercles was (25.0 ± 6.9)%, (32.5 ± 7.4)% and (42.5 ± 7.8)%, respectively, and a significant difference between these indices was not found, which indicates their redistribution due to the increase in the number of long tubercles, as a result of the alveolar process preparation to eruption of other permanent molars, and short tubercles due to the fact that other permanent molars were erupting or have already erupted.

Comparing the maxillary tubercles on the right and the left sides in each child, we can conclude that in the first two age groups, the difference between the size and shape of the right and left tubercles is not observed. However, at the age of 11-12 years, in 75.0% of the examined children, the shape and size of the maxillary tubercles differ from each other, which can be associated with nonsimultaneous eruption of other permanent molars on both sides. Thus, in 9 children (22.5% of the examined in this group) we

observed a combination of short and long maxillary tubercles, which was due to the nonsimultaneous eruption of other permanent molars. In 19 children (47.5%) medium tubercles on one side and long tubercles on the other side were found, and only 2 children (5.0%) had a combination of short and medium tubercles.

Thus, when studying the features of maxillary tubercles structure, we proposed the distribution of the maxillary tubercles, which are divided by shape into flat, sloping and convex; by height – into low (up to 2 mm), medium (2-3 mm) and high (over 3 mm); by length – into short (up to 5 mm), medium (5-8 mm) and long (over 8 mm).

The study on the prevalence of maxillary tubercles of different shapes and sizes in all age groups revealed that during the mixed occlusion period due to the growth of the jaw and the alveolar process, the tubercles tend to increase in height and elongation. Given the age of the child and individual features of the dental system's development, we can assume the possibility of using high, long and convex maxillary anchor systems.



Fig. 4. Orthodontic appliance for distalization of molars in the model.

The base band covers the maxillary tubercles, which acts as a support for the distal movement of the molar, moving along the arc, which contains a loop in the distal part, which is connected to the molar attachment by means of an elastic ligature (fig. 4). The appliance is activated once a week. Using our proposed appliance, high and convex maxillary tubercles can be used as a self-sustained anchor system, as well as in combination with other anchor systems.

It is known that for bodily distal movement of molars a number of fixed appliances designs with an anchor in the form of the Nance appliance are most frequently used (pendulum

appliance, distal jet, Jones-Jig, sliding mechanics, first class appliance) [5, 15], as well as distalizers, when used which anchor system are implants, alone or in combination with known anchor systems [6]. However, when using Nance “cushion” appliances as a medial support for molar movement, many authors point out that when they are used, the actual distalization amounts 70%, while the loss of the anchor due to proclination of incisors and angulation of molars accounts for 30% [11, 13].

To reduce the anchor loss and to eliminate the protraction of the frontal area during distalization of the molars, we considered the possibility of using the maxillary tubercles as a distal support for the distal movement of the molars. After analyzing the size and shape of the maxillary tubercles, we proposed an orthodontic appliance for distalization of molars, which permits both to use the maxillary tubercles as the main anchor system and to combine different types of systems when moving the first permanent molars. In addition, when using this appliance there is almost no protraction of the frontal group of teeth, and the mesial displacement of the first premolar is much smaller than in using appliances with mesial support and is corrected for further orthodontic treatment.

Conclusions

1. The features of the maxillary tubercles structure in children are studied and their distribution by shape is proposed: a) flat; b) sloping; c) convex; by height: a) low (up to 2 mm); b) medium (2-3 mm); c) high (more than 3 mm); by length: a) short (up to 5 mm); b) medium (5-8 mm); c) long (more than 8 mm).

2. In the mixed occlusion period there is a change in the shape of the maxillary tubercles from sloping to convex, increasing their height and elongation.

3. It is established that high, long and convex maxillary tubercles can both be used as the main anchor system, and to combine different types of systems to move the first permanent molars.

4. Запропоновано ортодонтичний апарат для дисталізації, конструкція якого дає можливість використовувати високі, довгі та випуклі горби верхньої щелепи в якості як основної якорної системи, так і поєднувати різні види систем для переміщення перших постійних молярів.

4. An orthodontic appliance for distalization is proposed, which design permits both to use high, long and convex maxillary tubercles as the main anchor system and to combine different types of systems to move the first permanent molars.

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Реферати

**МОРФОМЕТРИЧНИЙ АНАЛІЗ ГОРБІВ
ВЕРХНЬОЇ ЩЕЛЕПИ У ДІТЕЙ
ДЛЯ ВИЗНАЧЕННЯ МОЖЛИВОСТІ
ЇХ ВИКОРИСТАННЯ В ЯКОСТІ ОПОРИ
ПРИ ДИСТАЛІЗАЦІЇ**

Заяць О.Р., Ожоган З.Р., Ожоган І.А.

Висока інтенсивність та поширеність каріозного процесу призводять до передчасного видалення молочних зубів та створення сприятливих умов для розвитку аномалій та деформацій. З метою підвищення ефективності лікування зубощелепних аномалій, які виникають унаслідок патологічного мезіального зміщення бічних зубів верхньої щелепи, нами розглянута можливість використання горбів верхньої щелепи як самостійної або додаткової дистальної опори для переміщення постійного моляра в дистальному напрямі. Унаслідок вивчення особливостей будови горбів верхньої щелепи нами запропонований їх розподіл за формою : а) на плоскі; б) пологі; в) випуклі; за висотою: а) на низькі (до 2 мм); б) середні (2-3 мм); в) високі (понад 3 мм); за довжиною: а) короткі (до 5 мм); б) середні (5-8 мм); в) довгі (понад 8 мм), вивчено поширеність різних за розмірами та формою горбів верхньої щелепи у дітей різних вікових груп, а також запропоновано ортодонтичний апарат для дисталізації молярів із використанням високого випуклого горба верхньої щелепи, як дистальної опори.

Ключові слова: діти, горби верхньої щелепи, патологічне мезіальне зміщення перших молярів, якрна система, дисталізація молярів.

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**МОРФОМЕТРИЧЕСКИЙ АНАЛИЗ БУГРОВ
ВЕРХНЕЙ ЧЕЛЮСТИ У ДЕТЕЙ
ДЛЯ ОПРЕДЕЛЕНИЯ ВОЗМОЖНОСТИ
ИХ ИСПОЛЬЗОВАНИЯ В КАЧЕСТВЕ ОПОРЫ
ПРИ ДИСТАЛИЗАЦИИ**

Заяць А.Р., Ожоган З.Р., Ожоган И.А.

Высокая интенсивность и распространенность каріозного процесса приводят к преждевременному удалению молочных зубов и образованию благоприятных условий для развития аномалий и деформаций. С целью повышения эффективности лечения зубочелюстных аномалий, возникающих вследствие патологического мезиального смещения боковых зубов верхней челюсти, нами рассмотрена возможность использования бугров верхней челюсти как самостоятельной или дополнительной дистальной опоры для перемещения постоянного моляра в дистальном направлении. В результате изучения особенностей строения бугров верхней челюсти нами предложено их распределение по форме: а) на плоские; б) наклонные; в) выпуклые; по высоте: а) на низкие (до 2 мм); б) средние (2-3 мм); в) высокие (более 3 мм); по длине: а) короткие (до 5 мм); б) средние (5-8 мм); в) длинные (более 8 мм), изучено распространенность различных по размерам и форме бугров верхней челюсти у детей разных возрастных групп, а также предложено ортодонтический аппарат для дистализации моляров с использованием высокого выпуклого бугра верхней челюсти, как дистальной опоры.

Ключевые слова: дети, бугры верхней челюсти, патологическое мезиальное смещение первых постоянных моляров, якорная система, дистализация молярів.

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THERMAL CYCLING AS A METHOD FOR DISINTEGRATION OF BIFIDOBACTERIUM BIFIDUM

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The study compares the damaging effects of two thermal cycling methods on *Bifidobacterium bifidum* probiotic strain. The suspensions of freshly isolated bifidobacteria and of bacteria stored under hypothermic conditions for 24 hours were subject to the two methods of tenfold thermal cycling that involved slow cooling to $(-23 \pm 1)^\circ\text{C}$ or rapid cooling to $(-196 \pm 1)^\circ\text{C}$ followed by thawing at 37°C . Cell survival was assessed by means of colony forming units (CFU) counting and by flow cytometry using carboxyfluorescein diacetate (cFDA) as fluorochrome. Thermal cycling with slow cooling to $(-23 \pm 1)^\circ\text{C}$ had a more pronounced disintegrating effect on bifidobacteria. Preliminary storage of bifidobacteria under hypothermic conditions did not significantly increase their resistance to the disintegrating effect of thermal cycling.

Key words: bifidobacteria, thermal cycling, survival, flow cytometry, disintegration.

The study is a fragment of the research project: "Microbiological characteristic of new structural and metabolic complexes of lacto- and bifido- probiotics", state registration No. 0119U100686.

Studies of the low temperatures effects on bifidobacteria have been lasting for several decades. The main efforts were aimed at the development of technologies for long-term storage of bifidobacteria at low temperatures, ensuring their morphological, physiological and genetic stability. Development of long-term storage technologies is caused by their application in health care [11, 12, 14]. Today, the main methods for long-term storage of bifidobacteria are cryopreservation and lyophilization [11]. Optimal conditions that ensure the maximum survival and preservation of bifidobacteria functional activity during cryopreservation and lyophilization have been determined owing to the long-term studies of the of individual cryodamage factors influence on cell viability [3, 4, 5].

In vivo studies have shown that the therapeutic effects of cellular probiotics, especially their lyophilic forms, are obscure, short time or absent. First of all, the lack of probiotics efficacy is associated with their significant loss during their passage through the gastrointestinal tract and with low ability of probiotic cells to colonize the intestinal mucosa. Secondly, some patients also have a specific colonization resistance to introduced probiotics [15]. Survival of various strains of probiotic microorganisms in the gastrointestinal tract is about 20–40 % [1]. All the above mentioned necessitates the search for alternative approaches to the correction of microecological disturbances. One of these approaches is the use of probiotic derivatives: structural components and metabolites of probiotics.

Isolation of the bacterial structural components is quite difficult. One of the methods for obtaining bacterial structural components is freezing-thawing. Lactic acid bacteria tolerate repeated freeze-thaw cycles by immersion in liquid nitrogen [2, 10]. Each cycle results in loss of sensitive cells but more resistant ones remain. Therefore, cell disintegration by freezing-thawing is usually accompanied by other physical or mechanical methods [2].

Bacteria of the *Bifidobacterium* genus have significant biotechnological potential. For example, they can serve as a source of various biologically active structural components and metabolites [10]. Bifidobacteria belong to gram-positive bacteria. Their cell wall is stronger than that of many gram-negative bacteria and confers high mechanical stability. Bifidobacteria are also highly resistant to damaging factors that take place during freezing. High cryoresistance of bifidobacteria is explained by the presence of peptidoglycan and high content of phospholipids and unsaturated fatty acids in their cell membranes. However, there are differences in cryosensitivity among representatives of the same species. *B. bifidum* is more sensitive than other representatives that are protected by the polysaccharide capsule [3].

There is a number of factors, which may destroy cells during cooling. Rapid cooling increases the risk of cell damage due to intracellular crystallization. On the other hand, slow cooling leads to loss of water, which reduces the risk of intracellular crystallization but increases the negative impact of concentrated solutions on biological structures called "solution effects". Crystallization and recrystallization exacerbate the damage of microbial cells due to low-temperature, osmotic problems and oxidative stress. According to previously published data, the optimal freezing rate of bifidobacteria is $1^\circ\text{C}/\text{min}$, while plunging in liquid nitrogen in saline solution has the greatest damaging effect on cells [6, 7]. The possibility of using the low-temperature as a destructive factor for probiotic cells necessitates the search for freezing parameters that can help to obtain the maximal yield of biologically active derivatives.

The purpose of the study was to compare the damaging effects of the two thermal cycling methods on *B. bifidum* probiotic strain to determine the best approach to their disintegration.

Materials and methods. The probiotic strain *B. bifidum* (of medical product Bifidumbacterin, JSC Vivo-Actyv, Ukraine), stored in a lyophilized state at $(6 \pm 2)^\circ\text{C}$, was rehydrated and cultured for 20–24 hours at temperature $(37 \pm 1)^\circ\text{C}$ in Thioglycol medium (Biolife, Італія). After checking the purity of the culture, the microbial mass was washed three times with 0.9% sodium chloride solution to remove components of the nutrient medium. Cell suspensions with an optical density (OD) of 10.0 units by McFarland scale in saline solution ($\sim 10^9$ CFU/ml) were prepared using a Densi-La-Meter device (Lachema, Czech Republic). The resulting suspensions were subjected to cooling immediately after preparation. Other suspensions were stored under hypothermic conditions $(6 \pm 2)^\circ\text{C}$ before cooling for 24 hours. Cooling of the microbial suspensions was carried out in the following ways:

A – samples in 100 ml vials were placed into the cooling camera at $t = (-23 \pm 1)^\circ\text{C}$. The volume of each sample comes to 50 ml;

B – 4 ml samples in 4.5 ml cryocontainers were cooled by direct plunging into liquid nitrogen up to the $t = (-196 \pm 1)^\circ\text{C}$.

The frozen samples were warmed in a water bath at $(37 \pm 1)^\circ\text{C}$ until complete thawing. Such freeze-thaw cycles were repeated 10 times. Changes in the sample temperature during freezing-thawing were recorded using a copper-constantan thermocouple placed in the center of the sample.

Microscopic examination of native bifidobacteria suspensions preparations was carried out using the MPI-5 interference-polarization microscope (produced by PZO, Poland) equipped with digital camera with on-line access to a computer. The cell size and the concentration were determined with graduated ocular reticulum.

The number of colony forming units (CFU) represents the number of viable microorganisms. Serial dilutions method was used to determine the concentration of viable bacteria in samples prior and after the thermal cycling. 1 ml of test sample was dissolved in 9 ml of saline solution and vortexed until the sample was completely dissolved. From this first dilution, 10-fold serial dilutions (10^{-2} – 10^{-9}) were prepared in saline solution. Appropriate dilutions were cultured in two parallel tubes with high columns of Bifidum medium (BM, "Pharmaktiv", Ukraine) to obtain a reasonable number of colonies for counting. The tubes were incubated at $(37 \pm 1)^\circ\text{C}$ for 48 hours. The number of CFU per ml of suspension was calculated by the formula: $[(A \cdot 10^x) + (B \cdot 10^y)]/2$, (where A is the average number of colonies in the dilution preceding the largest one (10^{-x}), B is the average number of colonies in the largest dilution (10^{-y}) and expressed as a decimal logarithm (lg CFU/ml).

The viability of bifidobacteria was determined by cytofluorimetry using carboxyfluorescein diacetate (cFDA) as fluorochrome. The stock solution (10 mM) of cFDA was prepared by dissolving 4.6 mg of cFDA/ml in acetone and stored at 20°C in the dark. Next, the working solution was prepared by dissolving the stock solution in acetone to a concentration of 1 mM. Samples containing 10^3 – 10^7 cells/ml were incubated in phosphate-buffered saline supplemented with $10\ \mu\text{M}$ cFDA for 30 min. Then the samples were kept on ice in the dark for no longer than 1 h before flow cytometry. After that these samples were analyzed by flow cytometer FACSCalibur (Becton Dickinson Immunocytometry Systems, San Jose, USA).

All experiments were performed three times. The average values (M) and standard deviations (SD) were determined. The data were statistically processed using Excel 2010 software (Microsoft, USA). One-way analysis of variance (ANOVA) and subsequent multiple comparisons were performed using the Bonferroni correction. The differences between the obtained indices were considered statistically significant at $P < 0.05$.

Results of the study and their discussion. The mean cooling rate was low ($\sim 0.2^\circ\text{C}/\text{min}$) when method A was used. After 4 hours of cooling the final temperature of $(-23 \pm 1)^\circ\text{C}$ was achieved. Direct plunging in liquid nitrogen by method B allowed to cool the samples to $(-196 \pm 1)^\circ\text{C}$ in 2 minutes. The mean cooling rate was $\sim 109^\circ\text{C}/\text{min}$.

Fig. 1 demonstrates bifidobacteria before (1a and 1b) and after (2a and 2b) disintegration by method A. In the samples that were not subjected to thermal cycling some cells had the shape of straight or slightly curved rods, 0.5 – 1.2×1.5 – 8 microns in size, with club-shaped bulges at their ends. These cells were located individually. Other cells had V- or Y-like shapes. They tended to form clusters occupying the entire field of view. As a result of thermal cycling, the shape, size and arrangement of these cells changed. Their mean size decreased. The cells acquired a grain-like shape typical for cocci form. They were located evenly across the field of view. The presence of different sizes cell aggregates was peculiar to thermally cycled bacteria.

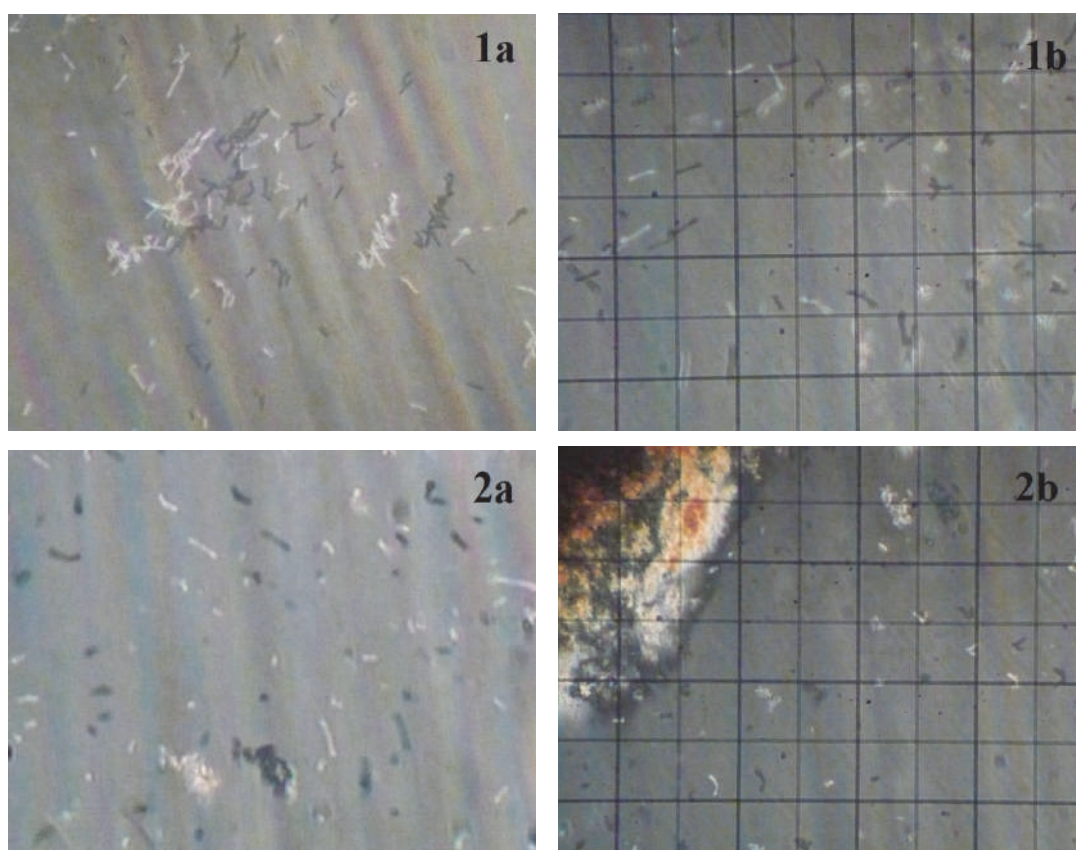


Fig. 1. Interference-polarization microscopy of bifidobacteria before (1a and 1b) and after (2a and 2b) disintegration by method A.

CFU counting allowed estimation of cell survival after the thermal cycling (table 1). Survival of bifidobacteria depended on the freeze-thaw regimen. The survival rate of bifidobacteria thermocycled by methods A and B came to 35 % and 53 %, respectively. Survival of bifidobacteria stored at hypothermic conditions before cooling did not significantly differ from that of freshly isolated bacteria. Thus, method A had a more pronounced damaging effect on bifidobacteria, so it was more suitable for disintegration of bifidobacteria.

Table 1

Survival of *B. bifidum* after thermal cycling (TC)

Method of TC	Concentration of survived cells, lg CFU/ml, M±SD	
	F	H
A	3.3±0.3	3.5±0.4
B	5.0±0.2*	5.3±0.3*

Notes: F – freshly isolated cells; H – cell stored in hypothermic conditions; * – the differences are statistically significant with respect to method A, $p < 0.05$.

Flow cytometry is a modern and efficient tool for viability of lactic acid bacteria [6]. Carboxyfluorescein diacetate (cFDA) is a non-fluorescent precursor, which upon entering the cell is converted into carboxyfluorescein (cF) by cell esterases [13]. It can fluoresce and is unable to penetrate membranes and leave cells unless they are damaged. Cells with intact membranes and functioning cytoplasmic enzymes are capable of holding the dye. Dead cells do not fluoresce because they lose enzymatic activity and cF through damaged membranes.

In order to compare the damaging effects of these two thermal cycling methods on bifidobacteria, flow cytometry with cFDA was applied. Additionally, freshly isolated bacteria were killed by heating to 70 °C for 30 min to be used as controls. Freshly isolated, killed and thermally cycled by methods A and B bacteria differed in their ability to be stained with fluorochrome (cF) and to scatter direct light. Within each experimental group the following cell subpopulations can be distinguished (fig. 2):

- 1) right top – undamaged cells of normal size with preserved esterase activity and membrane integrity, stained by cF;
- 2) left top – small undamaged cells stained by cF;
- 3) right bottom – cells of normal size that were not stained by cF. They are dead cells, damaged cells that have lost or inhibited esterase activity, impaired integrity or permeability of membrane;
- 4) left bottom – small dead or damaged cells and cellular debris (cell fragments, microparticles and microvesicles).

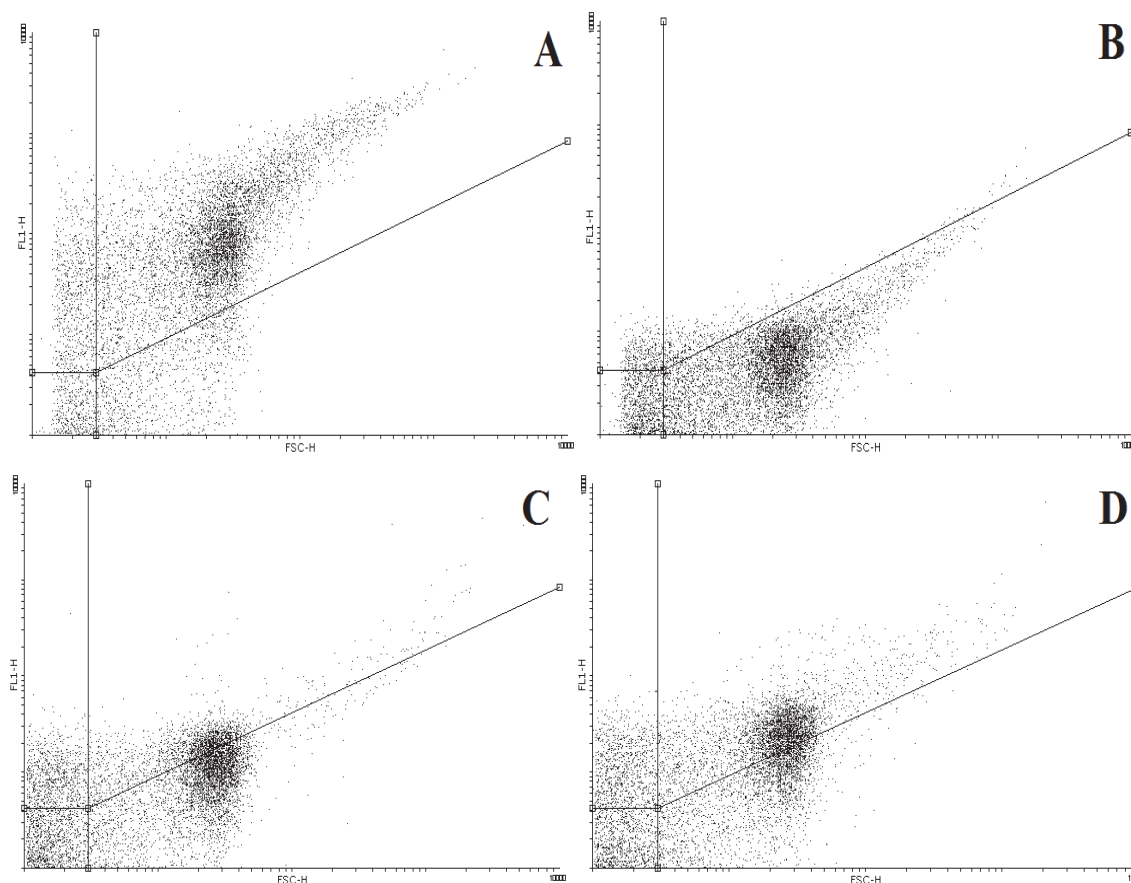


Fig 2. Dual-parameter dot plot of the forward scatter intensity versus cF-fluorescence of: A – freshly isolated; B – heat-killed; C – freshly isolated cells, thermally cycled by method A; D – freshly isolated cells, thermally cycled by method B.

Flow cytometry also showed that thermal cycling by method A caused more severe cell damage than by method B (table 2). The cells thermally cycled by method A had higher damaged /undamaged cells ratio comparing to method B. The data support the assumption put forward earlier that method A is more suitable for disintegration of bifidobacteria.

Flow cytometry revealed that samples previously stored under hypothermic conditions, after thermal cycling had a slightly lower damaged/undamaged cells ratio compared to freshly isolated samples. However, these differences in damaging effect were not significant.

Table 2

Subpopulation composition of freshly isolated, killed, and thermally cycled bifidobacteria

	Quadrants	Subpopulation content (%) in cell suspensions				
		A	B	C	D	E
1	Right top	60.22	3.94	18.11	36.38	20.09
2	Left top	7.55	3.20	8.82	9.74	9.27
3	Right bottom	17.65	73.09	46.39	29.86	44.76
4	Left bottom	14.58	19.77	26.68	24.02	25.88
Damaged /undamaged cells ratio		0.47	13.0	2.71	1.17	2.41

Notes: A – freshly isolated cells; B – heat-killed cells; C – freshly isolated cells, thermally cycled by method A; D – freshly isolated cells, thermally cycled by method B; E – stored in hypothermic conditions cells, thermally cycled by method A.

Previous studies showed that the use of saline solution as a cell medium during freezing of bifidobacteria led to more significant loss of cells than the use of cultural media or media with cryoprotectants. In the case of freezing in saline solution, the survival of bifidobacteria depended on the cooling rate. Cell survival was maximum when the cooling rate was 1 °C/min and decreased when the cooling rate became greater or less than 1 °C/min [3, 4]. That is why in the present study we used slow (~ 0.2 °C/min) and rapid (~ 109 °C/min) cooling rates.

The results of the study showed that ten-fold thermal cycling caused significant, but not complete loss of viable cell. The greatest loss of bifidobacteria and higher content of damaged and dead cells were observed after thermal cycling by method A. Thus, it can be stated that “solution effect” was the main factor that disintegrated bifidobacteria. Rapid cooling by method B did not have such a profound effect

on cell damage as in the case of method A. Therefore, the cells were better-tolerated higher cooling rates and intracellular crystallization that often accompanies such type of cooling. The results of the study are in good agreement with the data of other authors, who claim that "rapid" cooling or direct immersion in liquid nitrogen is accompanied by minimal loss of bacterial viability [2, 10]. Therefore, in the industrial production of probiotics, direct immersion of concentrated starter cultures in a cryoprotective medium is used [8]. Our data confirm that the damage to bacterial cells is due to osmotic imbalance rather than the formation of intracellular ice. It is known that the highest indicators of quantitative and functional losses of microorganisms are observed in the temperature range from $-40\text{ }^{\circ}\text{C}$ to $-60\text{ }^{\circ}\text{C}$ [7]. There is a certain critical limit of dehydration, the excess of which results in the death of bacterial cells due to inactivation and damage to critical sites in the cell - membrane lipids, proteins, nucleic acids, and some enzymes. Damage to the membranes and cell walls of bacteria as a result of thermal cycling leads to the release of cellular content into the surrounding. Consequently, the thermally cycled samples of bifidobacteria contain living cells, dead cells, cellular debris and material released from the destroyed cells after thermal cycling. In the present study, the cell disintegration efficiency was proved by determining the contents of living cells, dead cells, cellular debris in the samples. As far as the main purpose of disintegration is to obtain cellular derivatives, further identification of bifidobacteria derivatives in the extracellular media is necessary to confirm its efficacy. In addition, it is important to confirm that probiotic derivatives do not lose their biological activity during thermal cycling. These works are the next stage of the study on probiotic derivatives obtained by thermal cycling.

Conclusions

1. Both methods of thermal cycling caused significant cell damage of *B. bifidum*. However, they did not lead to complete loss of viable cells.
2. Method A that involved slow cooling to $(-23 \pm 1)\text{ }^{\circ}\text{C}$ had a more pronounced disintegrating effect on bifidobacteria than method B that involved rapid cooling to $(-196 \pm 1)\text{ }^{\circ}\text{C}$.
3. Preliminary storage of bifidobacteria under hypothermic conditions did not significantly raise their resistance to the disintegrating effect of thermal cycling.

Prospects of further research: the data obtained in this work will be taken into account and used in subsequent studies of the biological activity of B. bifidum derivatives.

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Реферати

**ТЕРМОЦИКЛЮВАННЯ ЯК МЕТОД
ДЕЗИНТЕГРАЦІЇ BIFIDOBACTERIUM BIFIDUM**
**Книш О.В., Пахомов О.В., Погоріла М.С.,
Савінова О.М., Балак О.К.**

У дослідженні порівнюється пошкоджуючий вплив двох методів термоцилювання на пробіотичний штам *B. bifidum*. Суспензії свіжовиділених біфідобактерій і бактерій, що зберігалися за гіпотермічних умов протягом 24 годин, піддавали десятикратному термоцилюванню двома способами, які передбачали повільне охолодження зразків до $(-23 \pm 1)^\circ\text{C}$ або швидке охолодження до $(-196 \pm 1)^\circ\text{C}$ з подальшим відігріванням на водяній бані при 37°C до повного відтавання. Вживання клітин оцінювали шляхом підрахунку колонієутворюючих одиниць і проточної цитометрії з використанням карбоксифлуоресцеїну діацетату (кФД) як флуорохрому. Термоцилювання з повільним охолодженням до $(-23 \pm 1)^\circ\text{C}$ виявило більш виражену дезінтегруючу дію на біфідобактерії. Попереднє зберігання біфідобактерій за гіпотермічних умов не значно підвищувало їх стійкість до дезінтегруючої дії термоцилювання.

Ключові слова: біфідобактерії, термоцилювання, виживаність, проточна цитометрія, дезінтеграція.

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**ТЕРМОЦИКЛИРОВАНИЕ КАК МЕТОД
ДЕЗИНТЕГРАЦИИ BIFIDOBACTERIUM BIFIDUM**
**Кныш О.В., Пахомов А.В., Погорелая М.С.,
Савинова Е.М., Балак А.К.**

В исследовании сравнивается повреждающее действие двух методов термоциклирования на пробиотический штамм *B. bifidum*. Суспензии свежeweделенных бифидобактерий и бактерий, хранившихся в гипотермических условиях в течение 24 часов, подвергали десятикратному термоциклированию двумя способами, которые предполагали медленное охлаждение образцов до $(-23 \pm 1)^\circ\text{C}$ или быстрое охлаждением до $(-196 \pm 1)^\circ\text{C}$ с последующим отогревом на водяной бане при 37°C до полного оттаивания. Выживаемость клеток оценивали путем подсчета колониеобразующих единиц и проточной цитометрии с использованием карбоксифлуоресцеина диацетата (кФД) в качестве флуорохрома. Термоциклирование с медленным охлаждением до $(-23 \pm 1)^\circ\text{C}$ оказывало более выраженное дезинтегрирующее действие на бифидобактерии. Предварительное хранение бифидобактерий в гипотермических условиях не значительно повышало их устойчивость к дезинтегрирующему действию термоциклирования.

Ключевые слова: бифидобактерии, термоциклирование, выживаемость, проточная цитометрия, дезинтеграция.

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**MORPHOLOGICAL FEATURES OF DOXORUBICIN-INDUCED LIVER DAMAGE
ASSOCIATED WITH NONALCOHOLIC STEATOHEPATITIS**

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The paper considers the study of histological features of anthracycline-induced liver lesions concomitant with non-alcoholic steatohepatitis. The findings of the study have established the presence of moderate fatty degeneration of the liver with mild focal protein dystrophy of hepatocytes in the lobules of the animals with experimental non-alcoholic steatohepatitis. In the group of animals with anthracycline-induced liver damage, moderate periportal necrosis of hepatocytes along with a mild small-droplet fatty degeneration. Prominent total (centrilobular and periportal) subacute liver necrosis along with moderate fatty degeneration was found in animals with anthracycline-induced liver damage associated with experimental non-alcoholic steatohepatitis.

Keywords: non-alcoholic steatohepatitis, anthracycline-induced liver damage, rats.

The work is a fragment of the research project "Development of methods for prevention and treatment of the drug-induced damages of the internal organs", state registration No. 0115U001087.

Doxorubicin belongs to anthracycline antibiotics that are considered as one of the most effective antitumor drugs widely used in oncological and oncohematological clinical practice [5, 6, 9-11]. Doxorubicin is the most important mainstay in the treatment of breast cancer, soft tissue sarcoma and aggressive lymphomas of high malignancy, acute lymphoblastic and myeloblastic leukemias [6, 9-11]. In some cases, high toxicity of doxorubicin may be restriction on its use [5, 6, 8-11]. Moreover, the toxic effect of doxorubicin on the tissues of the heart, kidneys, liver has been confirmed [5, 9, 12-14]. Notably, damaging effect of anthracycline antibiotics is due to its specific pharmacokinetics. Doxorubicin is able to accumulate intracellularly in concentrations 10-500 times higher than extracellularly [8]. Another important point in the development of toxic effects of all cytostatics, including anthracyclines, is the impact on all cells, both malignant and healthy [7, 8]. Doxorubicin is metabolized mainly in the liver with the formation of a highly toxic metabolite of doxorubicinol, which has a direct damaging effect on liver tissue [6]. Histological manifestations of liver lesions induced by anthracycline antibiotics are characterized by necrosis and degeneration of hepatocytes, sinus dilatation, vascular stagnation and hemorrhage [6, 11, 13, 14].

Over the last decades experimental and clinical studies have been conducted to study the mechanisms of the organotoxic effect of doxorubicin. Thus, D. Chaudhary et al. [6] reported about the study of doxorubicin hepatotoxicity performed on 60 albino mature rats administered with doxorubicin at a single dose of 10 mg/kg body weight. Anatomical and histomorphological parameters were studied on day 7 and 14. Liver weight in rats treated with doxorubicin on day 14 of the study was significantly lower than in the control group. The diameter of hepatocytes, the size of the nucleus of the hepatocyte was greater both on day 7 and 14 compared to the control group ($p < 0.001$). Thus, the authors proved that the toxic effect of doxorubicin is delayed and increases with time. Similar changes in the histological structures of the liver were observed by a number of researchers who confirmed that doxorubicin causes the enlargement of hepatocytes and their nuclei, as well as the change in the shape of the hepatocyte nucleus and disruption of their membrane structure [6, 11, 13, 14].

We believe that the study of histomorphological features of liver lesions in the presence of concomitant nonalcoholic steatohepatitis (NASH), which may contribute to drug metabolism and potentiate the vulnerability of hepatocytes to the toxic effects of cytostatics is crucial [3, 4].

The purpose of the work was to study histological features of anthracycline-induced liver lesions concomitant with NASH.

Materials and methods. The study involved 30 mature outbred albino rats (male rats=15 (50%); female rats=15 (50%) weighted 160-220 g. The study was carried out in two stages.

At the first stage 10 rats (5 males and 5 females) were exposed to modeled NASH during 9 weeks (from day 1 to day 63), induced by a diet, containing 42,8% fats. The daily ration per one animal included combination fodder-concentrate granulated 0.04 kg, 72.5% butter 0.01 kg, refined sunflower oil 0.01 kg, palm oil 0.01 kg. Vegetables were excluded from the ration of the experimental animals. 4% aqueous solution of fructose was used as the sole source of liquid. Another 20 rats received a regular rations of vivarium, containing combination fodder-concentrate granulated 0.04 kg, low-fat cheese 0.006 kg, carrots 0.02 kg, cabbage 0.015 kg per one animal a day for 9 weeks (from day 1 to day 63).

At the second stage of the experiment the modeling of doxorubicin- induced liver damage was carried out. Experimental rats were divided into 3 groups:

Rats of Group I (n=10; males n=5; females n=5) were exposed to modeled NASH, induced by a high-calorie diet from day 1 to day 63, followed by administration of 5mg/kg/day doxorubicin intraperitoneally for 3 days (from day 64 to day 66) to reach the cumulative doze of 15 mg/kg;

Rats of Group II (n=10; males n=5; females n=5) were on a regular rations of vivarium from day 1 to day 63, followed by administration of 5mg/kg/day doxorubicin intraperitoneally from day 64 to day 66 to reach the cumulative doze of 15 mg/kg;

Rats of Group III (n=10; males n=5; females n=5) were on a regular rations of vivarium from day 1 to day 63, followed by administration of intraperitoneal 0.9% sodium chloride solution at a dose of 1 ml for 3 days.

Euthanasia of rats was performed under 50 mg/kg thiopental anesthesia on day 67 of the experiment. The obtained fragments of the liver were fixed in buffered 10 % formalin for 24 hours that were subsequently embedded into paraffin according to conventional technique [1]; sections of 5 μ m thick were made and stained with hematoxylin and eosin.

Animal housing and experiments on them have been carried out in compliance with the principles of the “European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes” (Strasbourg,1986), “General Ethic Rules for Conducting Experiments on Animals”, adopted by the I National Congress on Bioethics [2] and the requirements of the “Procedure for conducting tests, experiments on animals by research institutions” (2012).

The study and documentation of the sections was carried out using the Biorex–3 BM–500T microscope in $\times 400$ magnification, equipped with the DCM–900 digital microphoto attachment and software adapted for the above studies.

Results of the study and their discussion. The analysis of the histological sections of the liver of rats of the control group has found that it had a lobular structure. Central veins were detected in the center of each lobule. Hepatic beams formed by hepatocytes and sinusoidal capillaries between them were visualized radially. In the central zones of the lobules, binuclear hepatocytes were determined on the average 8.36 ± 0.08 in the field of view (fig. 1a).

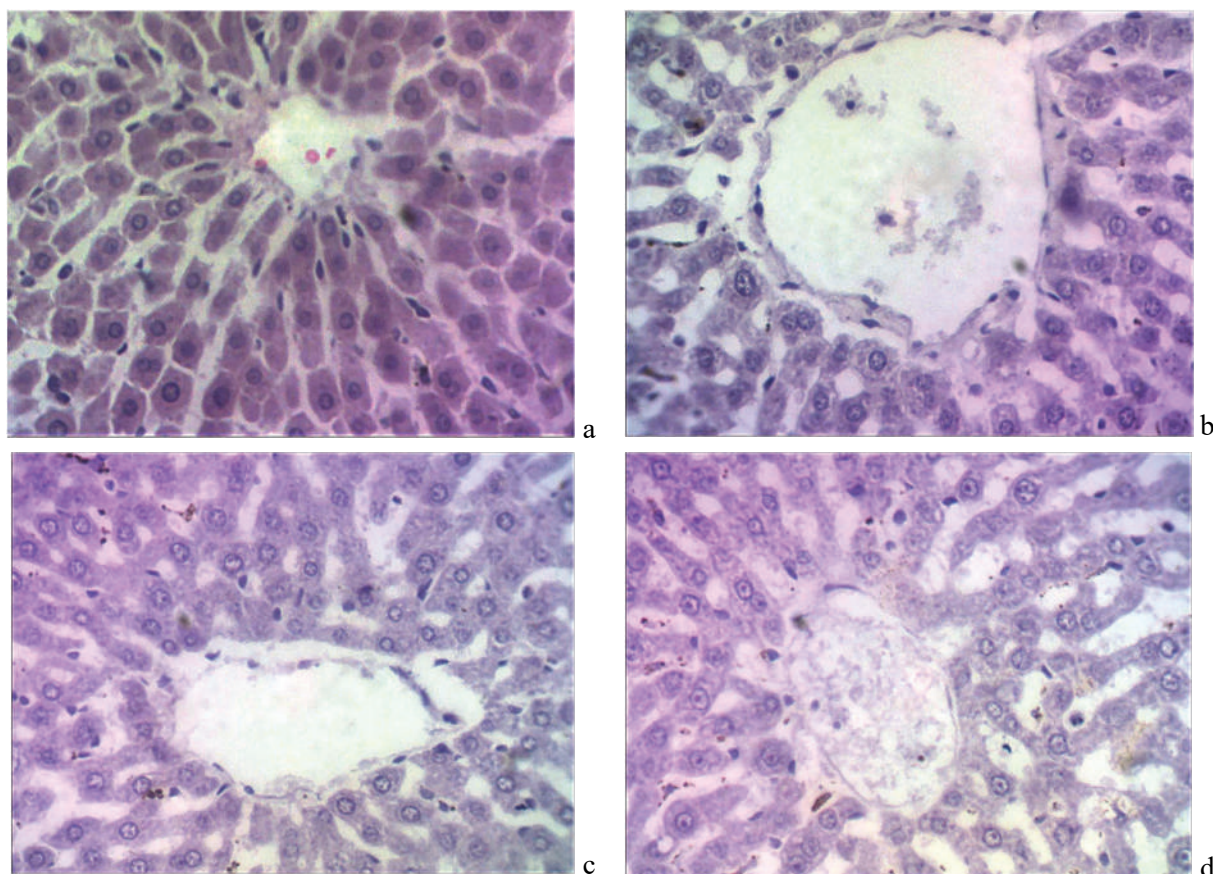


Fig. 1. Central part of the liver lobule of rat of control group (a), with experimental non-alcoholic steatohepatitis (b), with anthracycline-induced liver damage (c) and with anthracycline-induced liver damage associated with experimental non-alcoholic steatohepatitis (d). H&E stain. Lens: $\times 40$ magnification; Ocular lens: $\times 10$ magnification.

In rats with simulated experimental nonalcoholic steatohepatitis, varicose central veins were detected in the central areas of the lobules. The endothelium was thinned; the nuclei of endothelial cells were visualized in the form of thin basophilic stripes. Cellular detritus and sporadic squamous cells were noted in the lumens. Signs of amorphous hyperhydration were detected perivascularly. Sinusoidal capillaries were dilated with heterogeneous blood supply. Hepatocytes showed polymorphism and moderate manifestations of fatty degeneration. Binuclear cells were not visualized. Hepatocytes with pyknotically altered nuclei were detected locally (fig. 1b).

The study of histological sections of the liver of rats with anthracycline-induced liver damage has found that the central veins were dilated with morphological signs of edema, detected perivascularly. Sinusoidal capillaries were unevenly dilated and ischemic (fig. 1c).

The beam-radial structure of the lobules was preserved, but cells with pyknotically altered nuclei and karyorhexis phenomena, as well as with small-droplet fatty degeneration, were detected. The number of binuclear hepatocytes decreased compared to the control group of animals, accounting for 3.04 ± 0.02 in the field of view.

In the central parts of the lobules of the liver of rats with anthracycline-induced liver damage associated with experimental non-alcoholic steatohepatitis the central veins were dilated, the endothelium was thinned. The lumens were filled with inhomogeneous content of medium optical density. The sinusoidal capillaries were significantly dilated. The nuclei of the vast majority of hepatocytes were visualized in the state of karyopyknosis, karyorhexis and karyolysis. The cytoplasm showed cytolysis along with moderate small-droplet fatty degeneration (fig. 1d).

In the intermediate parts of the lobules of control rats, the shape of the hepatocytes was more orbicular and larger than the cells in the central parts of the lobules. Binuclear cells were sporadic. Uncondensed chromatin predominated in the nuclei and single centric nucleolus was determined (Fig. 2a).

In rats exposed to simulated experimental nonalcoholic steatohepatitis, dilated sinusoidal capillaries with inhomogenous blood supply were detected in the intermediate zones of the lobules. Hepatocytes were polymorphic with hydropic and fatty degeneration. Binuclear cells were not visualized. Hepatocytes with the phenomena of karyopyknosis were detected (fig. 2b).

Histological sections of the intermediate parts of the liver lobules of rats with anthracycline-induced liver damage showed that the sinusoidal capillaries were inhomogenously dilated, locally narrowed, and ischemic.

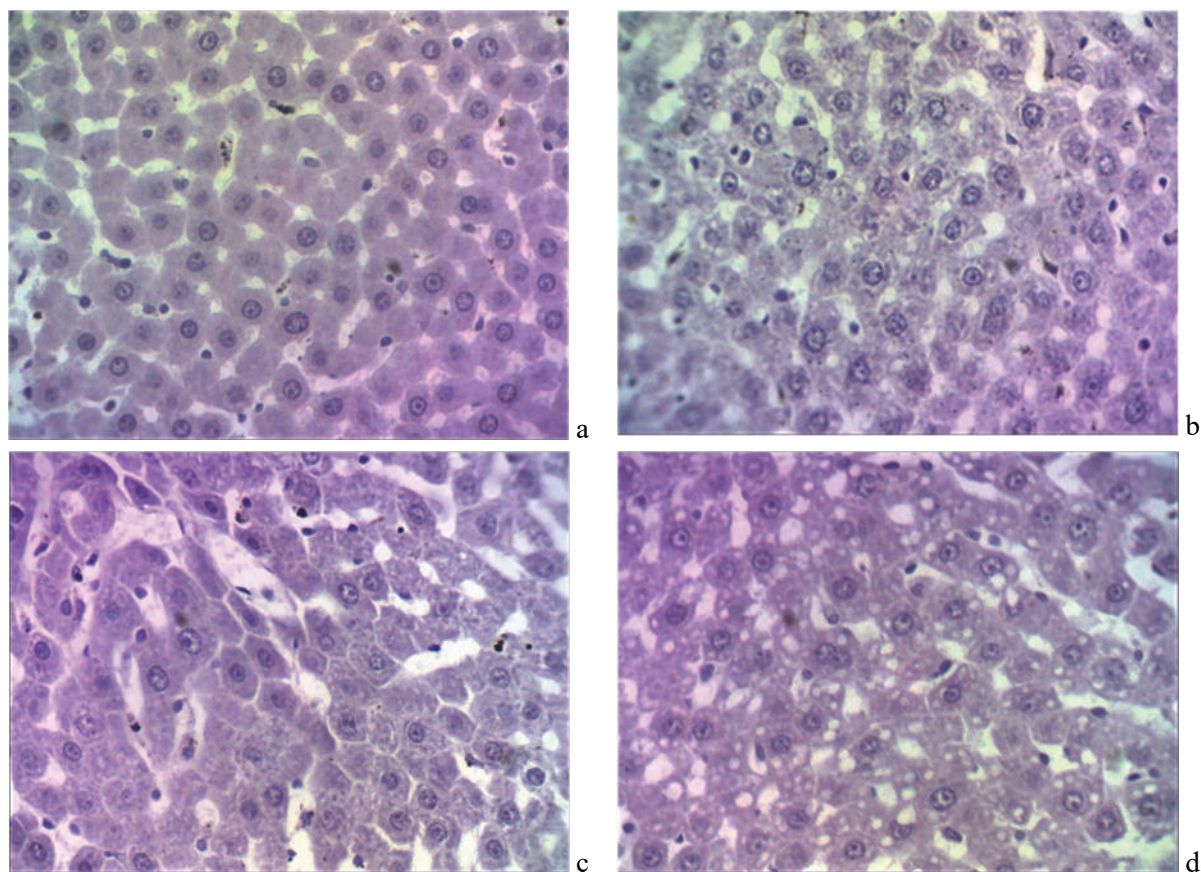


Fig. 2. Intermediate part of the lobule of the liver of rat of control group (a), with experimental non-alcoholic steatohepatitis (b), with anthracycline-induced liver damage (c) and with anthracycline-induced liver damage associated with experimental non-alcoholic steatohepatitis (d). H&E stain. Lens: $\times 40$ magnification; Ocular lens: $\times 10$ magnification.

The beam-radial structure of the lobules was preserved, but cells with a dark unstructured cytoplasm and compacted nuclei were detected. The vast majority of hepatocytes were with small-droplet fatty degeneration. Binuclear hepatocytes were not detected (fig. 2c).

In the intermediate parts of the lobules of the liver of rats with anthracycline-induced liver damage associated with experimental nonalcoholic steatohepatitis, sinusoidal capillaries were inhomogenously dilated; some of them were narrowed due to edema of hepatocytes, in the cytoplasm of which large vacuoles were found (fig. 2d).

Portal triads composed of the artery, vein and bile duct were localized on the periphery of the lobules in rats of the control group. The blood supply of the vascular system of the lobules was moderate. The number of binuclear hepatocytes was 2 ± 0.01 in the field of view. The vast majority of cells had a cubic shape (fig. 3a).

The study of histological sections of the liver of rats with experimental non-alcoholic steatohepatitis has found that in the peripheral parts of the liver lobules arterioles were spasmodic in the triads, nuclei of the endothelial cells protruded into the lumens. The surrounding connective tissue had morphological signs of hyperhydration of the amorphous substance and was infiltrated by leukocytes, namely, lymphocytes, macrophages and single segmented leukocytes. The sinusoidal capillaries were inhomogenously dilated and ischemic. Hepatocytes were shrunken, the nuclei of the latter were pyknotic; small-droplet fatty degeneration was noted in the cytoplasm (fig. 3b).

In the experimental animals with anthracycline-induced liver damage, hepatocytes with dark cytoplasm and elongated cells dominated in the peripheral parts of the lobules. Karyopyknosis and karyolysis were observed in some cells. In the triads arteries were spasmodic and poorly expressed periportal edema was detected. Leukocyte infiltrates, where macrophages and lymphocytes prevailed, were visualized periportal (fig. 3c).

The study of histological sections of the liver of rats with anthracycline-induced liver damage associated with experimental non-alcoholic steatohepatitis has shown edema of the periportal stroma and its infiltration by macrophages and plasma cells were detected on the periphery of the lobules. Karyorhexis and karyopyknosis were noted in the vast majority of hepatocytes, as well as cytolysis in the cytoplasm. The preserved cells were visualized in a state of small-droplet hydropic degeneration (fig. 3d).

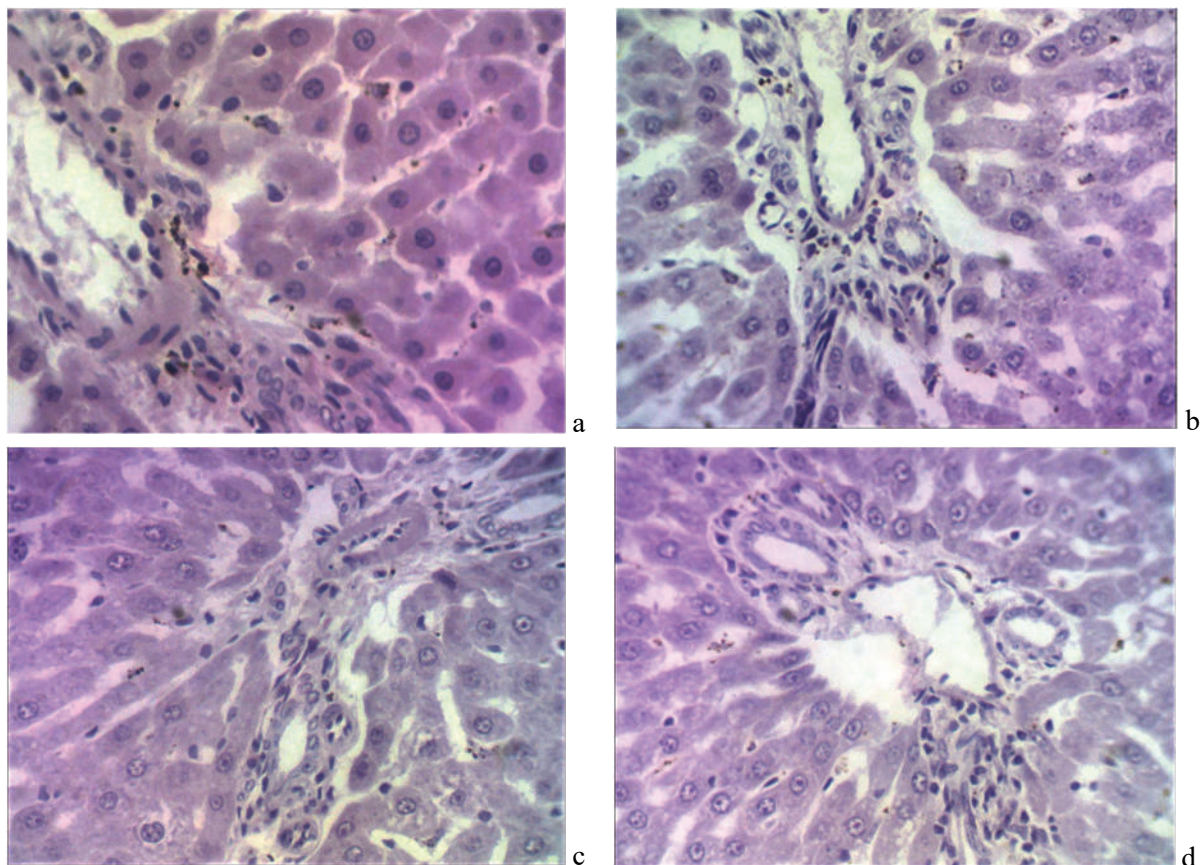


Fig. 3. Portal triad and peripheral portion of the liver lobule of the rat of control group (a), with experimental nonalcoholic steatohepatitis (b), with anthracycline-induced liver damage (c) and with anthracycline-induced liver damage associated with experimental nonalcoholic steatohepatitis (d). H&E stain. Lens:×40 magnification; Ocular lens:×10 magnification.

The findings of the study related to histofunctional changes in the liver in anthracycline-induced liver damage are consistent with the findings of other researchers [6, 11, 13, 14]. Non-alcoholic steatohepatitis contributes to the drug metabolism disorder, as well as potentiates the vulnerability of hepatocytes to the toxic effects of cytostatics [3, 4].

Conclusion

The findings of the study have established the presence of moderate fatty degeneration of the liver with mild focal protein dystrophy of hepatocytes in the lobules of the animals with experimental non-alcoholic steatohepatitis. In the group of animals with anthracycline-induced liver damage, moderate periportal necrosis of hepatocytes along with a mild small-droplet fatty degeneration. Prominent total (centrilobular and periportal) subacute liver necrosis along with moderate fatty degeneration was found in animals with anthracycline-induced liver damage associated with experimental non-alcoholic steatohepatitis.

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Реферати

МОРФОЛОГІЧНІ ОСОБЛИВОСТІ ДОКСОРУБІЦІН-ІНДУКОВАНИХ УРАЖЕНЬ ПЕЧІНКИ НА ТЛІ НЕАЛКОГОЛЬНОГО СТЕАТОГЕПАТИТУ

Маслова Г.С., Скрипник І.М., Єрошенко Г.А.

В роботі досліджено гістологічні особливості антрациклін-індукованих уражень печінки на тлі неалкогольного стеатогепатиту. В результаті проведеного дослідження встановлено, що у тварин з експериментальним неалкогольним стеатогепатитом у часточках печінки визначено помірно виражену жирову дистрофію печінки зі слабо вираженою осередковою білковою дистрофією гепатоцитів. В групі тварин з антрациклін-індукованим ураженням печінки встановлений помірний перипортальний некроз гепатоцитів на тлі слабо вираженої дрібнокрапельної жирової дистрофії. Виражений тотальний (центролобулярний і перипортальний) підгострий некроз печінки на тлі помірно вираженої жирової дистрофії виявлений у тварин з антрациклін-індукованим ураженням печінки на тлі експериментального неалкогольного стеатогепатиту.

Ключові слова: неалкогольний стеатогепатит, антрациклін-індуковані ураження печінки, щурі.

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МОРФОЛОГИЧЕСКИЕ ОСОБЕННОСТИ ДОКСОРУБИЦИН-ИНДУЦИРОВАННОГО ПОРАЖЕНИЯ ПЕЧЕНИ НА ФОНЕ НЕАЛКОГОЛЬНОГО СТЕАТОГЕПАТИТА

Маслова Г.С., Скрипник И.М., Ерошенко Г.А.

В работе исследованы гистологические особенности антрациклин-индуцированных поражений печени на фоне неалкогольного стеатогепатита. В результате проведенного исследования установлено, что у животных с экспериментальным неалкогольным стеатогепатитом в долях печени выявлено умеренно выраженную жировую дистрофию печени со слабо выраженной очаговой белковой дистрофией гепатоцитов. В группе животных с антрациклин-индуцированным поражением печени установлен умеренный перипортальный некроз гепатоцитов на фоне слабо выраженной мелкокапельной жировой дистрофии. Выраженный тотальный (центролобулярный и перипортальный) подострый некроз печени на фоне умеренно выраженной жировой дистрофии обнаружен у животных с антрациклин-индуцированным поражением печени на фоне экспериментального неалкогольного стеатогепатита.

Ключевые слова: неалкогольный стеатогепатит, антрациклин-индуцированные поражения печени, крысы.

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REACTION OF HEMOMICROCIRCULATORY BED OF RAT LIVER AND CHANGES IN THE FUNCTIONAL STATE OF THE NITRIC OXIDE CYCLE UNDER THE CONDITIONS OF MODELING ALCOHOLIC HEPATITIS

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The purpose of the work was to study the changes in the hemomicrocirculatory bed of the liver and the role of the NO-ergic system in their development under the conditions of modeling alcoholic hepatitis. At the early stages of modeling alcoholic hepatitis, the thickness of the vascular wall of the central vein, interlobular artery and lobular arterioles increases, while the thickness of the vascular wall of the interlobular vein, the lobular venule and the sublobular vein decreases. These changes are associated with dysregulatory changes in the nitric oxide cycle in rat liver. Dysregulatory changes are manifested by an increase in the activity of inducible and constitutive isoforms of NO synthases against the background of decreased activity of arginases in the absence of statistically significant changes in the activity of nitrate and nitrite reductases in the liver of rats with simulated alcoholic hepatitis.

Key words: liver, alcoholic hepatitis, nitric oxide cycle, rats.

The work is a fragment of the research project “Peculiarities of pathological changes development in digestive system organs and development of their correction methods”, state registration No. 0120U100502.

Alcohol consumption is the seventh leading risk factor for various diseases, injuries and death. In 6.8% of deaths among men and 2.2% among women, the cause is alcohol abuse. The total cost of eliminating the social consequences of alcohol consumption makes more than 1% of the gross national product for high- and middle-income countries, much higher than the budget for health care [5].

Alcohol consumption is a major cause of liver disease, and alcoholic hepatitis is a major chronic alcohol-related disease. Worldwide, per capita alcohol consumption is strongly correlated with mortality from liver cirrhosis [5]. It is estimated that alcohol abuse is the causative factor in 60 types of diseases and injuries and the simultaneous cause of at least in 200 other ones [8].

Despite the significant importance of the problem and the large number of publications devoted to the study of alcoholic hepatitis, the molecular mechanisms of alcoholic liver disease remain poorly understood. The role of nitric oxide (NO) in inflammation of the liver remains ambiguous because NO is reported to have both pro-inflammatory and anti-inflammatory properties [4]. The amount of NO, time and place of its synthesis are important factors of its biological impact. Among the nitric oxide-producing enzymes, the inducible isoform of NO synthase has a wide range of effects. New evidences emerge and lead to conclusion that the inducible isoform of NO synthase plays a key role in the initiation and development of liver tumors, which are a frequent complication of alcoholic hepatitis [2].

The development of alcoholic hepatitis is inextricably linked with the response of the liver hemomicrocirculatory bed. Vascular tone, capillary permeability directly depend on the NO-ergic system of the liver. Therefore, the study of changes in the NO-ergic system in the model of alcoholic hepatitis and its impact on the resistant, metabolic and capacitive links of the liver hemomicrocirculatory bed is a topical issue today.

The purpose of the work was to study the changes in the hemomicrocirculatory bed of the liver and the role of the NO-ergic system in their development under the conditions of modeling alcoholic hepatitis.

Materials and methods. The experiments were performed on 30 white adult Wistar male rats weighing 180-220 g. Animals were divided into 2 groups: group I – the control (n = 6); group II - animals with simulated alcoholic hepatitis (n = 24). We simulated alcoholic hepatitis by the method of forced intermittent alcoholism for 5 days, with repetition after two days by intraperitoneal administration of 16.5% ethanol solution in 5% glucose solution, at the rate of 4 ml / kg body weight [7]. The control group included animals that were subjected to similar manipulations throughout the study, but injected saline. Conditions for keeping animals in the vivarium were standard. Removal of animals from the experiment occurred on days 1, 3, 5 and 7 by taking blood from the right ventricle of the heart under thiopental anesthesia. Serum and liver were studied. During the experiments, the recommendations of the “European Convention for the protection of vertebrate animals used for experimental and other scientific purposes” were followed (Strasbourg, 1986). Experiments were conducted in accordance with the “General Principles of Animal Experiments” approved by the First National Congress of Bioethics, and the requirements of the "Procedure for conducting scientific experiments, experiments on animals" (2012).

In the serum of rats we determined the activity of γ -glutamyltranspeptidase (γ -GTP) using a diagnostic kit, manufacturer NPP “Philisit-Diagnostics”.

The activity of inducible and constitutive isoforms of NO synthase [8], nitrite nitrate reductases and arginases was determined in rat liver homogenate [1].

Total NO-synthase (gNOS) activity was assessed by nitrite growth after incubation of 10% liver homogenate [1]. To determine the activity of constitutive NOS (cNOS) to the first aliquot was added 0.1 ml of 1% solution of aminoguanidine hydrochloride, extending the incubation time to 60 min [8]. The activity of inducible NOS (iNOS) was calculated by the formula: $iNOS = gNOS - cNOS$ [8].

Nitrate-nitrite reductase activity was evaluated by reducing the concentration of nitrites and nitrates after incubation of 10% liver homogenate [1]. Arginase activity was determined by the increase of L-ornithine in 10% rat liver homogenate after incubation in phosphate buffer solution in the presence of excess L-arginine [1].

For morphometric studies, the liver was fixed in 10% neutral formalin, poured into paraffin blocks, from which semi-thin sections were prepared, which were stained with hematoxylin and eosin. Morphometric examination and microphotography were performed using a microscope Biorex-3 BM-500T with digital microphoto nozzle DCM 900 with programs adapted for research data. We determined the thickness of the vascular wall (by subtracting from the value of the outer diameter of the inner diameter of the vascular wall) of central vein, interlobular arteries and veins, arterioles and venules of hepatic lobes and sublobular vein.

Statistical processing of the results of biochemical studies was performed using pairwise comparison using the nonparametric Mann-Whitney method. Processing of the results of the morphometric study was performed using one-way analysis of variance by the method of Kruskal-Wallis with the subsequent use of pairwise comparison according to the exact Mann-Whitney test and taking into account the correction for the multiplicity of comparisons according to Bonferroni. All statistical calculations were performed in Microsoft office Excel and its extension Real Statistics 2019. The difference was considered statistically significant if $p < 0.05$. Data in tables represented as mean \pm standard error of mean (M \pm m).

Results of the study and their discussion. Biochemical analysis of serum in rats, with simulated alcoholic hepatitis, showed an increase in the activity of γ -GTP on the 3rd and 5th day of the experiment,

by 2.06 and 1.7 times respectively, compared to the control ($p < 0.05$) and by 2.06 times on day 3 compared to the activity of γ -GTP on day 1 of the experiment ($p < 0.05$) (tab. 1). On day 7 of the experiment, the activity of γ -GTP in the serum of rats decreased by 1.56 times compared with the activity of γ -GTP on day 5 of the experiment ($p < 0.05$).

Table 1

Activity of γ -glutamyltranspeptidase in the serum of rats with experimental alcoholic hepatitis, $M \pm m$

Parameters	Groups of animals				
	Control	1st day	3rd day	5th day	7th day
Activity of γ -glutamyl-transpeptidase, $\mu\text{cat} / \text{l}$	0.67 \pm 0.04	0.67 \pm 0.05	1.38 \pm 0.16* [^]	1.14 \pm 0.09*	0.73 \pm 0.07 [^]

* - $p < 0.05$ compared to the control group of rats; [^] - $p < 0.05$ compared to the previous term of the experiment.

The activity of cNOS in the liver of rats, with simulated alcoholic hepatitis, on the 3rd, 5th and 7th day of the experiment was increased by 1.93, 1.15 and 1.67 times, respectively, compared to the control ($p < 0.05$) (tab. 2). On the 5th day of the experiment, the activity of cNOS in the liver of rats decreased by 1.68 times compared with the activity of cNOS on the 3rd day of the experiment ($p < 0.05$). On the 7th day of the experiment, the activity of cNOS in the liver of rats increased by 1.45 times compared to the activity of cNOS on the 5th day of the experiment ($p < 0.05$).

Table 2

Biochemical parameters in the liver of rats with experimental alcoholic hepatitis, $M \pm m$

Parameters, $\mu\text{mol} / \text{min per g of protein}$	Groups of animals				
	Control	1st day	3rd day	5th day	7th day
Activity of constitutive NO synthases	0.027 \pm 0.0003	0.052 \pm 0.007	0.052 \pm 0.004*	0.031 \pm 0.001* [^]	0.045 \pm 0.001* [^]
Inducible NO-synthase activity	0.16 \pm 0.02	1.07 \pm 0.15*	0.72 \pm 0.07*	0.14 \pm 0.02 [^]	0.85 \pm 0.05* [^]
Nitrite reductase activity	3.11 \pm 0.51	3.4 \pm 0.42	2.54 \pm 0.48	1.23 \pm 0.13*	4.98 \pm 0.62 [^]
Nitrate reductase activity	3.71 \pm 0.81	3.76 \pm 0.59	4.52 \pm 0.69	1.32 \pm 0.2* [^]	6.07 \pm 1.36 [^]
Arginase activity	1.8 \pm 0.1	1.69 \pm 0.53	0.22 \pm 0.01*	0.18 \pm 0.01*	0.53 \pm 0.01* [^]

* - $p < 0.05$ compared to the control group of rats; [^] - $p < 0.05$ compared to the previous term of the experiment.

The activity of iNOS in the liver of rats simulated alcoholic hepatitis on days 1, 3 and 7 of the experiment increased by 6.69, 4.5 and 5.31 times, respectively, compared to the control ($p < 0.05$). On day 5 of the experiment, the activity of iNOS in the liver of rats decreased by 5.14 times compared to the activity of iNOS on day 3 of the experiment ($p < 0.05$). On the 7th day of the experiment, the activity of iNOS in the liver of rats increased by 6.07 times compared to the activity of iNOS on the 5th day of the experiment ($p < 0.05$).

The activity of nitrite reductases in the liver of rats, which simulated alcoholic hepatitis, on the 5th day of the experiment was reduced by 2.53 times compared to the control ($p < 0.05$). On the 7th day of the experiment, the activity of nitrite reductases in the liver of rats increased by 4.04 times compared to the activity of nitrite reductases on the 5th day of the experiment ($p < 0.05$).

The activity of nitrate reductases in the liver of rats, which simulated alcoholic hepatitis, on the 5th day of the experiment was reduced by 3.42 times compared to the activity of nitrate reductases on the 3rd day of the experiment ($p < 0.05$).

The activity of arginases in the liver of rats, which simulated alcoholic hepatitis, on the 3rd, 5th and 7th day of the experiment was reduced by 8.18, 10 and 3.4 times, respectively, compared to the control ($p < 0.05$). On the 7th day of the experiment, the activity of arginases in the liver of rats increased by 2.94 times compared to the activity of arginases on the 5th day of the experiment ($p < 0.05$).

A morphometric study of the hemomicrocirculatory bed of the liver of rats, with simulated alcoholic hepatitis, revealed that the thickness of the vascular wall of the central vein of the rats' hepatic lobe increased by 1, 3 and 7 days of the experiment, respectively, by 2.19, 2.04 and 1.39 times compared to the control ($p < 0.05$) (tab. 3). On the 5th day of the experiment, the thickness of the vascular wall of the central vein of the rats' hepatic lobe decreased by 1.76 times compared to the thickness of the vascular wall on the 3rd day ($p < 0.05$).

The vascular wall thickness of the interlobular artery of the liver of rats, with simulated alcoholic hepatitis, increased on the 1st, 3rd and 5th days of the experiment by 1.28, 1.54 and 1.51 times respectively, compared to the control ($p < 0.05$). On day 7 of the experiment, the vascular wall thickness of the interlobular artery of rats decreased by 1.59 times compared to the vascular wall thickness on day 5 ($p < 0.05$).

The vascular wall thickness of the hepatic lobe arterioles in rats, which were simulated alcoholic hepatitis on the 3rd, 5th and 7th day of the experiment increased by 2.28, 2.54 and 2.54 times respectively, compared to the control ($p < 0.05$). On day 3 of the experiment, the vascular wall thickness of the hepatic lobe arterioles of rats increased by 1.83 times compared to the vascular wall thickness on the 1st day ($p < 0.05$).

Morphometric parameters of the hemomicrocirculatory bed of rats with experimental alcoholic hepatitis, M±m

Parameters, μm	Groups of animals				
	Control	1st day	3rd day	5th day	7th day
Vascular wall thickness of the central vein	1.13±0.11	2.47±0.12*	2.3±0.15*	1.31±0.07^	1.57±0.09*
Vascular wall thickness of the interlobular artery	3.48±0.06	4.46±0.26*	5.37±0.06*	5.27±0.12*	3.32±0.12^
Vascular wall thickness of the lobular arteriole	0.69±0.06	0.86±0.05	1.57±0.15*^	1.75±0.1*	1.75±0.11*
Vascular wall thickness of the lobular venule	2.26±0.15	1.39±0.07*	1.04±0.08*	1.11±0.03*	1.08±0.07*
Vascular wall thickness of the intelobular vein	4.77±0.21	1.51±0.1*	1.79±0.07*	3.34±0.23*^	2.2±0.14*^
Vascular wall thickness of the sublobular vein	4.83±0.17	2.85±0.08*	2.71±0.35*^	2.12±0.09*	1.96±0.07*

* - $p < 0.05$ compared to the control group of rats; ^ - $p < 0.05$ compared to the previous term of the experiment.

The vascular wall thickness of the hepatic lobe venules of rats, with simulated alcoholic hepatitis, decreased on the 1st, 3rd, 5th and 7th day of the experiment by 1.63, 2.17, 2.04 and 2.09 times respectively, compared to the control ($p < 0.05$).

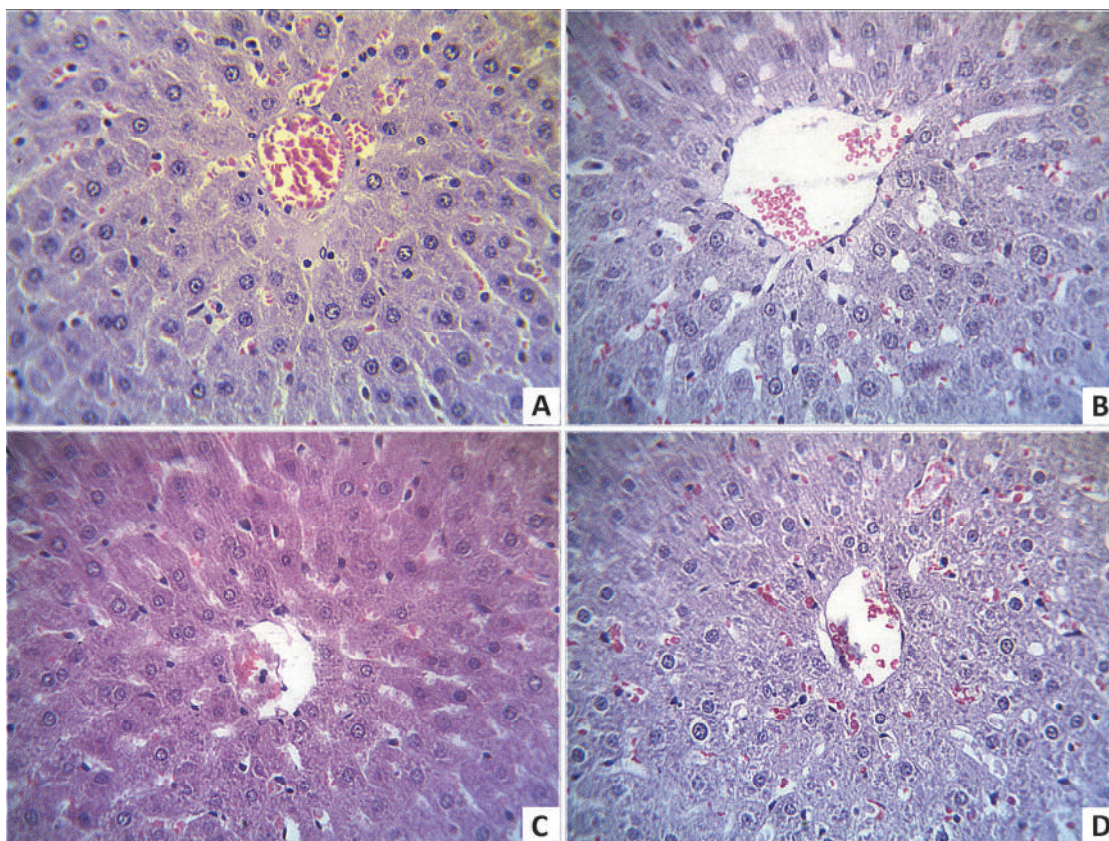
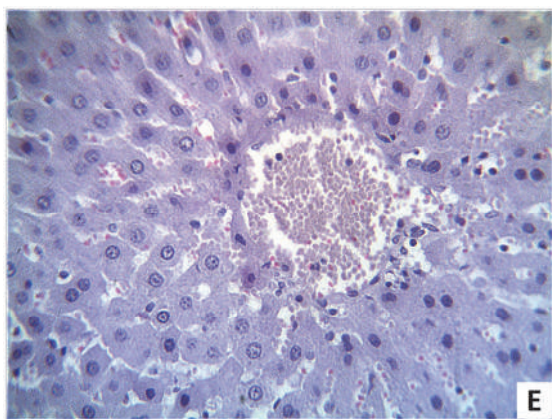


Fig. 1. Central vein of rat liver under conditions of alcoholic hepatitis simulation. Magnification: Lens x 40, Eyepiece x 10. A – control group of animals; B – 1st day of experiment; C – 3rd day of experiment; D – 5th day of experiment; E – 7th day of experiment.



The vascular wall thickness of the liver interlobular vein of rats with simulated alcoholic hepatitis, decreased on the 1st, 3rd, 5th and 7th day of the experiment by 3.16, 2.66, 1.43 and 2.17 times respectively, compared to the control ($p < 0.05$). On the 5th day of the experiment, the vascular wall thickness of the rats' interlobular vein increased by 1.87 times compared to the vascular wall thickness on the 3rd day ($p < 0.05$). On the 7th day of the experiment, the vascular wall thickness of the rats' interlobular vein decreased by 1.52 times compared to the vascular wall thickness on the 5th day ($p < 0.05$).

The vascular wall thickness of the liver sublobular vein in rats with simulated alcoholic hepatitis, decreased on the 1st, 3rd, 5th and 7th day of the experiment by 1.69, 1.78, 2.28 and 1.22 times respectively, compared to the control ($p < 0.05$).

On the first day of modeling, alcoholic hepatitis destruction of hepatocytes does not occur, because γ -GTP activity is within the levels of the control group of animals. An increase in the vascular wall thickness of the central vein and the interlobular artery can be considered signs of damage to these vessels (fig. 1B). A decrease in the vascular wall thickness of the lobular venule and the interlobular and sublobular vein may occur in response to a pressure decrease in the capacitive link of the hemomicrocirculatory bed of the rats' liver (fig. 2B). A possible reason for the described changes on the first day of the experiment is the action of nitric oxide, produced by inducible NO synthase.

On the third day of modeling alcoholic hepatitis, we observed a cytolysis of hepatocytes as evidenced by the increase in the activity of γ -GTP in the blood serum of rats. The dynamics of changes in the vascular walls thickness of the studied vessels on the third day remains the same as on the first day (fig. 1C, 2C). The role of constitutive isoforms of NO-synthase in the increased production of nitric oxide in the rats' liver with simulated alcoholic hepatitis. Competition between NO synthases and arginases for the substrate increases and leads to a decrease in the activity of arginases in the rats' liver with simulated alcoholic hepatitis. A possible reason for the decrease in the activity of arginases in the rats' liver may also be alcohol-dependent change in the polarization of Kupffer cells by M1 phenotype through activation of NF- κ B transcription factor [2].

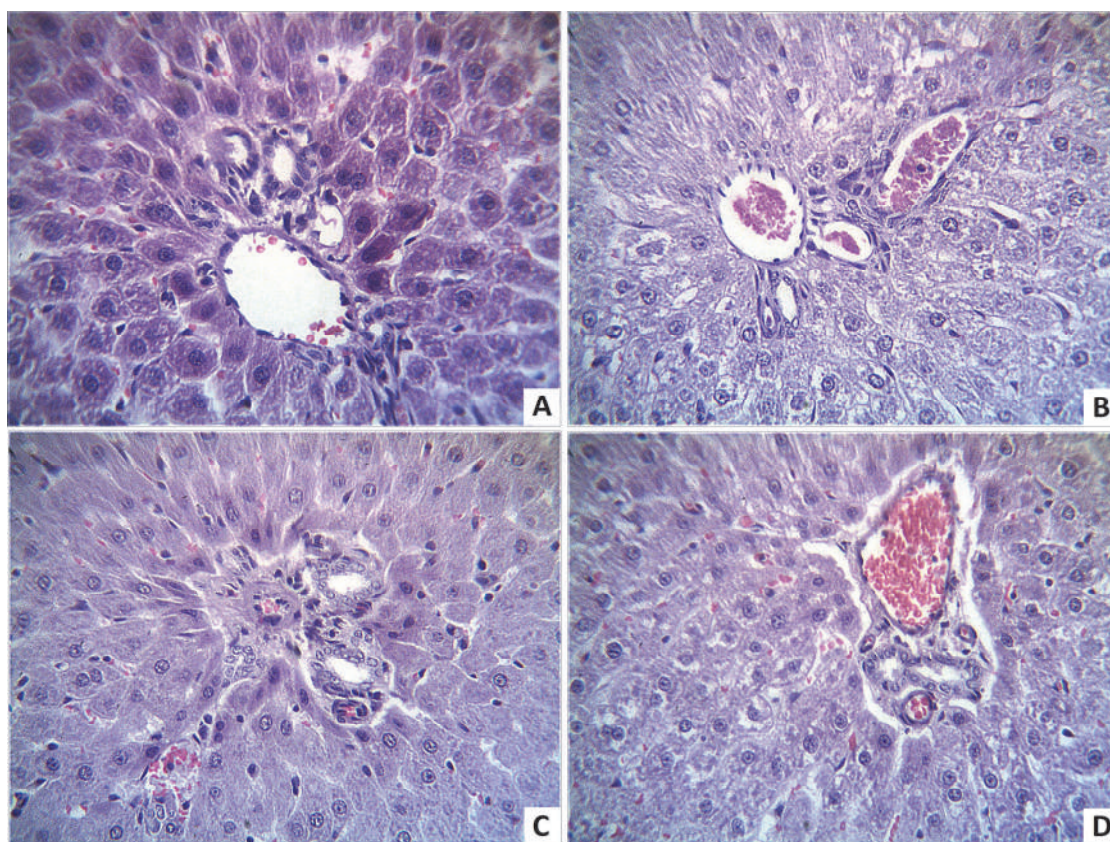
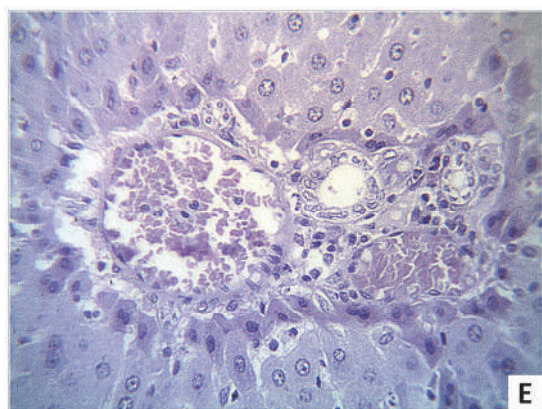


Fig. 2. Liver triad of rats under the conditions of modeling alcoholic hepatitis. Hematoxylin and eosin staining. Magnification: x 400. A – control group of animals; B – 1st day of experiment; C – 3rd day of experiment; D – 5th day of experiment; E – 7th day of experiment.



On the fifth day of modeling alcoholic hepatitis, the activity of γ -GTP in the blood serum of the rats' liver with simulated alcoholic hepatitis, remains at a high level. The vascular wall thickness of the central vein decreases, and that of the interlobular vein increases compared to the previous term of the experiment (fig. 1D, 2D). Production of nitric oxide in the rats' liver on the 5th day of modeling alcoholic hepatitis is reduced from all studied isoforms of NO synthase, as evidenced by the decrease in the activity of iNOS and cNOS to the level of the control group animals.

Nitrate-nitrite reductive mechanism of nitric oxide production in the rats' liver with simulated alcoholic hepatitis, also reduces its activity. Thus, in the rat liver on the 5th day of modeling alcoholic hepatitis there occurs a dysregulation of nitric oxide production, confirmed by a decrease in the activity of arginases, NO synthases and nitrate-nitrite reductases. A possible

cause of this phenomenon may be a deficiency of the reaction substrate for arginases and the deposition of nitric oxide, which was synthesized in large quantities at the previous stages of the experiment, in the form of nitrosothiols.

On the seventh day of modeling alcoholic hepatitis, the activity of γ -GTP in the blood serum of rats is reduced compared to the previous period of the experiment. The vascular wall thickness of the interlobular arteries and veins in the rats' liver decreases compared to the previous period of the experiment (fig. 1E, 2E). Production of nitric oxide in the liver of rats with simulated alcoholic hepatitis is increased by NO synthases and nitrite reductases. Arginases also increase their activity. These changes may be associated with an increase in the amount of substrate for NO synthases and arginases functioning in the rat liver, which is associated with the activation of autophagy. Autophagy, caused by alcohol, leads to increased protein catabolism. Short-term alcohol consumption increases the autophagy of mitochondria and damaged proteins, while activity of proteosomal protein degradation and lysosome formation decreases [3]. Prolonged alcohol consumption, on the other hand, reduces autophagy [3].

An important role in alcoholic liver damage is played by the macrophage link of the immune system. It is proved that the destruction of Kupffer cells by gadolinium chloride leads to a decrease in the intensity of inflammation in the conditions of alcoholic hepatitis [4]. There is a hypothesis that the transition of liver macrophages to the proinflammatory M1 phenotype during alcohol consumption is associated with damage to the intestinal mucosal barrier by ethanol when taken orally [5]. Damage to the intestinal mucosal barrier leads to increased entry of bacterial lipopolysaccharides into the liver, which provokes polarization of liver macrophages by M1 phenotype through Toll-like receptors type 4 [6].

However, the relevance of this hypothesis in our experimental model is questionable. This is due to the fact that our experimental model of alcoholic hepatitis involves intraperitoneal administration of ethanol in the experiment. Therefore, ethanol-dependent damage to the intestinal mucosa during this period is debatable. Studying the activity of marker enzymes of macrophage polarization (iNOS, arginase) we can indirectly claim the predominance of M1 polarization on the 3rd and 7th day of modeling alcoholic hepatitis in rats. On the first day of the experiment, the activity of iNOS increases, which may indicate the beginning of changes in the polarization of liver macrophages. On the fifth day of alcoholic hepatitis simulation, despite the fact that the activity of iNOS decreases to values in the control group of animals, it can be argued that rat liver macrophages are mostly in M1 state, because the activity of arginases is significantly reduced.

Taking into account the changes in marker enzymes of macrophage polarization (iNOS and arginase) and the features of the experimental model, we can note that the change in macrophage polarization is more associated with alcohol-dependent damage to hepatocytes than with alcohol-dependent damage to the intestinal mucosa.

Conclusion

An the early stages of modeling alcoholic hepatitis, the vascular wall thickness of the central vein, interlobular artery and lobular arterioles increases, while the vascular wall thickness of the interlobular vein, the lobular venule and the sublobular vein decreases. These changes are associated with disregulatory changes in the nitric oxide cycle in rat liver.

Disregulatory changes are manifested by an increase in the activity of inducible and constitutive isoforms of NO synthases against the background of the decreased activity of arginases in the absence of statistically significant changes in the activity of nitrate and nitrite reductases in the liver of rats with simulated alcoholic hepatitis.

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Реферати

**РЕАКЦИЯ ГЕМОМИКРОЦИРКУЛЯТОРНОГО
РУСЛА ПЕЧИНКИ ТА ЗМІНИ
В ФУНКЦІОНАЛЬНОМУ СТАНІ ЦИКЛУ
ОКСИДУ АЗОТУ ЗА УМОВ МОДЕЛЮВАННЯ
АЛКОГОЛЬНОГО ГЕПАТИТУ**

Микитенко А.О., Єрошенко Г.А.

Метою роботи було вивчити зміни гемомікроциркуляторного русла печінки та роль NO-ергічної системи в їх розвитку за умов моделювання алкогольного гепатиту. На ранніх термінах моделювання алкогольного гепатиту товщина судинної стінки центральної вени та міжчасточкової артерії і часточкової артеріоли збільшується, а товщина судинної стінки міжчасточкової вени та часточкової венули і підчасточкової вени зменшується, що пов'язано з дисрегуляторними змінами в циклі оксиду азоту в печінці шурів. Дисрегуляторні зміни полягають у збільшенні активності індукцибельної та конститутивних ізоформ NO-синтази на фоні зниження активності аргінази за відсутності статистично значущих змін в активності нітрат- та нітритредуктаз в печінці шурів, яким моделювали алкогольний гепатит.

Ключові слова: печінка, алкогольний гепатит, цикл оксиду азоту, шури.

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**РЕАКЦИЯ ГЕМОМИКРОЦИРКУЛЯТОРНОГО
РУСЛА ПЕЧЕНИ И ИЗМЕНЕНИЯ
В ФУНКЦИОНАЛЬНОМ СОСТОЯНИИ ЦИКЛА
ОКСИДА АЗОТА ПРИ УСЛОВИИ
МОДЕЛИРОВАНИЯ АЛКОГОЛЬНОГО ГЕПАТИТА**

Микитенко А.О., Єрошенко Г.А.

Целью работы было изучить изменения гемомікроциркуляторного русла печени и роль NO-эргической системы в их развитии в условиях моделирования алкогольного гепатита. На ранних сроках моделирования алкогольного гепатита толщина сосудистой стенки центральной вены и междольковой артерии и внутريدольковой артериолы увеличивается, а толщина сосудистой стенки междольковой вены, внутريدольковой венулы и поддольковой вены уменьшается, что связано с дисрегуляторными изменениями в цикле оксида азота печени крыс. Дисрегуляторные изменения заключаются в увеличении активности индукцибельной и конститутивных изоформ NO-синтазы на фоне снижения активности аргиназы при отсутствии статистически значимых изменений в активности нитрат- и нитритредуктаз в печени крыс, которым моделировали алкогольный гепатит.

Ключевые слова: печень, алкогольный гепатит, цикл оксида азота, крысы.

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**INFLUENCE OF PINEAL GLAND'S HYPOFUNCTION ON THE STRUCTURE
OF VISCERAL ORGANS**

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The purpose of this work was to study the morphological and functional changes in the pineal gland, heart, stomach, lungs and intestines of rats in the conditions of the pineal gland's hypofunction. The studies were carried out on 24 sexually mature male Wistar rats, which were kept in standard vivarium conditions with round-the-clock lighting for 30 days. As a result of the performed microscopic studies, it was found that the lack of melatonin is accompanied by erosive gastritis with atrophy of the glands, an increase in proliferative activity and in the number of pathological mitoses in the jejunum of rats, which may indicate the genesis of malignant tumors. Dystrophies, atrophy and hypertrophy of cardiomyocytes, foci of cardiomyocytes' lysis, circulatory impairment and inflammatory changes in lung tissues, which can be considered moderately expressed intestinal pneumonia, were revealed.

Key words: pineal gland, pinealocytes, hypofunction, cardiomyocytes, intestinal pneumonia, gastritis.

The work is a fragment of the research project "Features of metabolism and morphofunctional condition of visceral organs exposed to the influence of environmentally hazardous factors", state registration No. 0118U003395.

Among the physiological characteristics of living organisms, the fundamental one is the rhythm of their activity, which manifests itself in the periodicity of many functions, circadian rhythms, and seasonality [5, 9]. The pineal gland is considered to be the central link that provides the body with information about changes in the light regime. The secretion product of the pineal gland is the hormone melatonin, which regulates the body's biorhythms, both directly affecting cells and by changing the secretion of other hormones and biologically active substances, which concentration varies depending on the time of day [7, 8]. Various

changes in the synthesis of melatonin that go beyond physiological fluctuations can lead to disruption of the body's own biological rhythms between themselves or the rhythms of the body with the environment. The use of artificial lighting at night helps to change both the light regime and the duration of the light effect on the body. Such a violation of the photoperiod is becoming a part of the modern way and pace of life, which is accompanied by serious changes in the health status, which are manifested in the morphological and functional rearrangement of the organs in the visceral systems [2, 3, 4, 6]. In both cases, this is accompanied by the development of morphological and functional changes in tissues and diseases of internal organs.

The purpose of our work was to study the morphological and functional changes in the pineal gland, heart, stomach, lungs and intestines of rats in the conditions of the pineal gland's hypofunction.

Materials and methods. The study was carried out on 24 sexually mature male Wistar rats weighing 240 - 280 g. For the experiments, males were selected, since the level of melatonin in the blood plasma of females depends on the sexual cycle phase. The animals were kept under standard vivarium conditions. The hypofunction of the pineal gland was simulated by round-the-clock illumination with two fluorescent lamps with an intensity of 1000-1500 Lx, which were placed on both sides of the coop. The exposure of the animal organism to light has been carried out for 30 days [1]. The use of this model is due to several reasons. First, the use of other means, such as pinealectomy or blinding of animals, is accompanied by additional stress, which would undoubtedly affect the histological state of the organs under study and the correct interpretation of the results obtained. Secondly, melatonin is synthesized by the pineal gland only under darkroom conditions, and it is generally known that for white rats light intensity of 0.0005 mV / cm² is sufficient to reduce the production of melatonin. Therefore, this model, in our opinion, reproduces the state of hypomelatoninemia.

At the end of the experiment, the experimental animals were euthanized under light ether anesthesia in strict compliance with the provisions of the "European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes" (Strasbourg, 1986), as well as the "General Ethical Principles for Animal Experiments" adopted by the first national congress on bioethics (Kyiv, 2001).

After removing the organs under study, they were immersed in a fixing solution of 10% neutral formalin. Using standard methods, the material was embedded in paraffin blocks, from which sections were cut with the thickness of 4 μm and stained with hematoxylin and eosin. The histological slides obtained in this way were studied at various magnifications with "Carl Zeiss" microscope, followed by photographing the microslides with a "Canon G10 Wide" digital reflex camera.

Results of the study and their discussion. Microscopic analysis of the pineal gland parenchyma cellular composition revealed specific morphological changes. An increase in the number of inactive dark pineal cells, which are distinguished by an intense basophilic color, was established. Such cellular structures are located in the parenchyma of the pineal gland randomly, but in the immediate vicinity of the vascular wall's outer surface. As for light pinealocytes, they constitute the absolute majority, but it should be noted that the number of dark-nucleated light pinealocytes grows.

It was established that in most light cells, the nucleoli practically do not contour. This fact is explained by the excessive filling of the karyoplasm with basophilic substance, in the form of homogeneous structureless masses, which mask the cell nucleolus. This morphological feature indicates the transition of light active pinealocytes to the population of inactive dark cells, which, under conditions of increased functional activity, will pass into an active state. Thus, dark and light pineal cells are morphological reflections of various functional states.

It was revealed that in histological sections of the pineal gland parenchyma of some experimental animals, the phenomena of weakly expressed cytoplasm vacuolization in individual pinealocytes were noted. Such foci of pineal cells vacuolization are located mainly in the periphery, but sometimes they also occur in the central part of the pineal gland (fig. 1).

On the part of the pineal gland's vascular system, we also revealed characteristic morphological changes. So, in the lumen of intraorgan blood vessels, both subcapsular and those located in the central part of the pineal gland, disorders in the morphofunctional state of the blood formed elements were revealed. Structurally, this is expressed in the adhesion of erythrocytes with the formation of various conglomerates, which almost completely overlap the lumen of the respective blood vessel. The gaps between adjacent conglomerates of glued erythrocytes are filled with eosinophilic substance, which is a coagulated mass of plasma proteins. This ratio between various blood elements leads to impairment of its rheological properties, and to occurrence of hypertrophied endothelial cells in certain areas of the vascular wall (fig. 2). A similar reaction of endotheliocytes is accompanied by thickening of the wall and narrowing of the blood vessel's lumen. This phenomenon is especially pronounced in arterioles and precapillary arterioles.

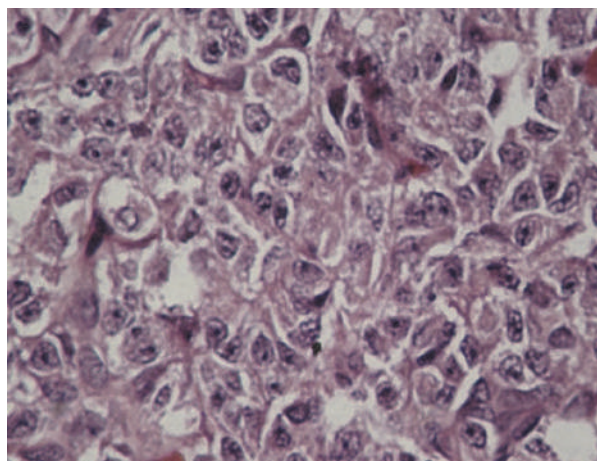


Fig. 1. Micrograph of the cellular composition of the rat pineal gland parenchyma under the conditions of prolongation by light. Magn.: oc. $\times 10$, ob. $\times 40$. Hematoxylin-eosin staining.

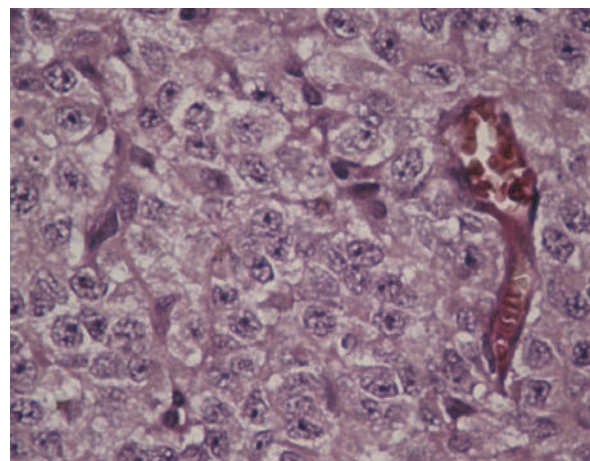


Fig. 2. Micrograph of rat pineal gland parenchyma under conditions of light prolongation. Magn.: oc. $\times 10$, ob. $\times 40$. Hematoxylin-eosin staining.

The histological picture of the heart in experimental animals is characterized by the presence of pronounced cardiomyocytes damage foci in the left ventricular myocardium. In the foci of granulation tissue development and in the lateral zone of normal myocardium, mitosis of connective tissue cells, uneven vascular blood filling, moderate perivascular edema, small hemorrhages, focal swelling and granularity of the intima in small myocardial arteries were often found. Microscopically, dilatation of capillaries and stasis, sludge phenomenon of erythrocytes, edema of interstitial tissue, perivascular hemorrhages, aggregations of leukocytes along the periphery of the ischemic zone were observed. Muscle fibers lose striation, glycogen, but are intensely stained with eosin. In the atrial myocardium, it is possible to reveal dystrophic changes in muscle fibers, areas of their significant deep degradation. Microscopic examination revealed a combination of dystrophy (protein, hydropic, fatty), atrophy and hypertrophy of cardiomyocytes, there were foci of cardiomyocyte lysis and sclerosis.

Limited foci of inflammatory infiltration were located subpericardially, and pronounced edema was observed. These changes can be assessed as a consequence of the fibrous pericarditis development. In the atrial myocardium, the occurrence of mitotic figures could be noted. In the muscles of the rat heart, where both muscle and stromal cells proliferate, one can note morphological differences in the mitoses

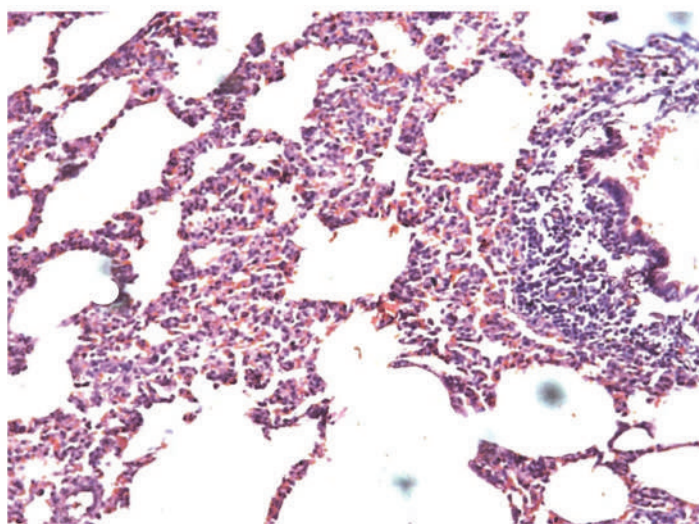


Fig. 3. Interstitial pneumonia in animals with 30-day pineal gland's hypofunction: 1 - capillary plethora; 2 - increased area of distelectases; 3 - bronchial obstruction; 4 - hyperplasia of lymphoid tissue; 5 - emphysema. Paraffin section. Magn.: ob. $\times 10$, oc. $\times 20$. Hematoxylin-eosin staining.

of these cells: connective tissue cells in the state of separation are, naturally, separated, their cytoplasm is basophilic, and the mitotic figure of myocytes is larger, the cytoplasm is lighter in significant areas, chromosomes are located within the muscle fiber. Most cells are in the state of mitosis, with half of all mitotic cells being in metaphase.

Under the prolonged illumination conditions, circulatory disorders in the form of capillary plethora with perivascularitis and interstitial edema were observed in rat lung tissue. In addition, inflammatory changes were found: with an increasing area of distelectases, bronchial obstruction with hyperplasia of lymphoid tissue was growing, bronchitis and interstitial alveolitis increased, which can be considered moderate interstitial pneumonia (fig. 3).

During the morphological study of the rat stomach histological slides, it was found that the mucous membrane is unevenly thinned, has micro-erosions, and is covered with desquamated epithelial cells (fig. 4 A). The proper lamina of the mucosa is expanded, has a moderately pronounced inflammatory infiltration. The stomach glands are of different sizes, most are cystic-enlarged and have atrophic changes. Both the

main and parietal cells have pale, narrow cytoplasm and small hyperchromic nuclei. The submucosal layer is expanded, has a swollen stroma.

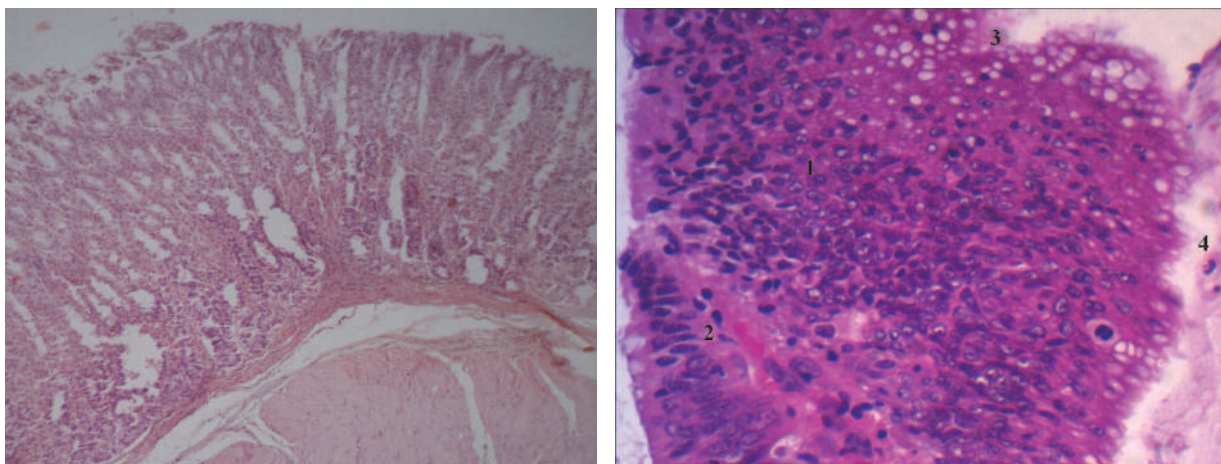


Fig. 4 A. Micrograph of rat stomach tissues with prolonged hypofunction of the pineal gland. Paraffin section. Magn.: ob. $\times 10$, oc. $\times 10$. Hematoxylin-eosin staining.

B. Distribution of cellular elements of rat jejunal epitheliocytes with a lack of melatonin. Magn.: ob. $\times 10$ oc. $\times 15$. Hematoxylin-eosin staining.

The muscle layer is thickened, muscle fibers are swollen, unevenly hypertrophied. In the submucosa, muscular and subserous membranes, moderately pronounced inflammatory, polymorphocellular infiltration is observed. The samples show chronic active erosive gastritis with glandular atrophy and thinning of the mucous membrane.

When studying histological preparations of the jejunum, it was established that intestinal crypts and villi had a somewhat smoothed appearance due to an increase in the number of mitotic undifferentiated cells. To a lesser extent, goblet cells produced mucus. At high magnification of the microscope, we noted the following features of the distribution of endocrinocytes: the cells had a well-developed cytoplasm, almost all of its surface was occupied by well-contoured nuclei. Nuclear-cytoplasmic ratio in some endocrinocytes was 0.8-0.9. Heterochromatin of the nuclei had a lumpy character and was located diffusely throughout the nuclear envelope. Nucleoli in the amount of 1-2, and sometimes more, were mainly localized along the periphery of the nuclei. In all fields of view of the microscope, cells were found that were in different phases of mitosis and were characterized by high proliferative activity (fig. 4 B).

Based on the distribution data of epithelial cells' proliferative activity, the mitotic index is 22.31%. A characteristic feature is that the overwhelming majority of cells were in a state of mitotic division, a large number of them being represented by pathological mitoses. Thus, the main pathology (16.7%) was predominantly K-mitosis with chromosome agglomeration, characterized by blockade of cell division in the metaphase as a result of damage to the mitotic apparatus. It is represented by hyperspiralized and thickened chromosomes, which form a dense conglomerate - a clump. The second place was occupied by a pathology of chromosome separation (9.6%), characterized by an uneven distribution of chromosomes in mitotic centers and a delay in their entry into daughter cells. In addition, a small number of bridges were observed, being the result of chromosome fragmentation with formation of a bridge, delaying and blocking the formation of cytotomy.

Numerous studies have shown that the experimental model of pineal gland hypofunction is accompanied by a decrease in melatonin levels in the blood plasma [9]. The results of our data indicate that in response to the effects of round-the-clock lighting morphofunctional destructive changes take place not only in the pineal gland, but also in the studied visceral organs, which according to literature sources indicate the development of specific pathological changes of inflammatory nature and consequent reduction of the visceral organs' functional activity [1, 6, 8].

Conclusion

The results of the histological slides histomorphological analysis indicate that the violation of the daily rhythm, that is, the change of day and night, is due to the stay of experimental animals in the conditions of a disturbed photoperiod for a long time, affects the morphological status and functional activity of not only the pineal gland but also nearly all visceral organs in animals.

1. In the pineal gland there is an adaptive morphofunctional restructuring of the parenchyma and the vascular bed in response to the prolonged exposure to light factors.

2. In the myocardium microslides, dystrophic changes and disturbances of the microcirculation bed, increased separation of connective tissue cells with the presence of K-mitosis are observed.

3. In the tissues of the lungs, destructive changes are observed, which are manifested by signs of interstitial pneumonia.

4. Manifestations of the pineal gland's hypofunction were also revealed in histological slides of the rat stomach, in the form of moderately pronounced chronic active erosive gastritis with the glands' atrophy and thinning of the mucous layer.

5. The revealed morphological changes in the jejunum indicate that the lack of melatonin sharply increases the number of pathological mitoses, which may indicate the presence of malignant tumors. At the same time, there is an increase in proliferative activity in the jejunum of rats.

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Реферати

ВПЛИВ ГИПОФУНКЦІЇ ЕПІФИЗА НА СТРУКТУРУ ВІСЦЕРАЛЬНИХ ОРГАНІВ Пшиченко В.В., Чеботар Л.Д., Ларичева О.М., Цвях О.О., Анасевич Я.М.

Метою даного дослідження було вивчення морфологічних і функціональних змін епіфіза, серця, шлунка, легень і кишечника шурів в умовах гіпофункції епіфізу. Дослідження проведені на 24 статевозрілих самцях шурів лінії Wistar, які перебували в стандартних умовах виварію в режимі цілодобового освітлення протягом 30 днів. В результаті проведених мікроскопічних досліджень встановлено, що нестача мелатоніну супроводжується ерозивним гастритом з атрофією залоз, збільшенням проліферативної активності і кількості патологічних мітозів в порожній кишці шурів, що може вказувати на появу злоякісних пухлин. Виявлено дистрофії, атрофії і гіпертрофії кардіоміоцитів, осередки лізису кардіоміоцитів, порушення кровообігу і запальні зміни в тканинах легень, які можна вважати помірною інтестиціальною пневмонією

Ключові слова: епіфіз, пинеалоцити, гіпофункція, кардіоміоцити, інтестиціальна пневмонія, гастрит.

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ВЛИЯНИЕ ГИПОФУНКЦИИ ЭПИФИЗА НА СТРУКТУРУ ВИСЦЕРАЛЬНЫХ ОРГАНОВ Пшиченко В.В., Чеботарь Л.Д., Ларичева А.Н., Цвях А.А., Анасевич Я.М.

Целью данного исследования было изучение морфологических и функциональных изменений эпифиза, сердца, желудка, легких и кишечника крыс в условиях гипofункции эпифиза. Исследования проведены на 24 половозрелых самцах крыс линии Wistar, которые находились в стандартных условиях вивария в режиме круглосуточного освещения в течение 30 дней. В результате проведенных микроскопических исследований установлено, что недостаток мелатонина сопровождается эрозивным гастритом с атрофией желез, увеличением пролиферативной активности и количества патологических митозов в тощей кишке крыс, что может указывать на появление злокачественных опухолей. Выявлены дистрофии, атрофии и гипертрофии кардиомиоцитов, очаги лизиса кардиомиоцитов, нарушение кровообращения и воспалительные изменения в тканях легких, которые можно считать умеренной интестициальной пневмонией

Ключевые слова: эпифиз, пинеалоциты, гипofункция, кардиомиоциты, интестициальная пневмония, гастрит.

Рецензент Єрошенко Г.А.

N.M. Seredynska, V.I. Korniyenko¹, K.S. Marchenko-Tolsta¹,O.M. Bobrytska¹, O.V. Ladohubets¹, K.A. Duchenko¹¹SI "Institute of Pharmacology and Toxicology NAMS of Ukraine", Kyiv¹Kharkiv State Zooveterinary Academy, Kharkiv, ²PHSEE "Kyiv Medical University", Kyiv**CARDIOTROPIC INFLUENCE OF SYNTHETIC AND GENETICALLY-ENGINEERED SUPPRESSORS IN RATS WITH EXPERIMENTAL RHEUMATOID ARTHRITIS COMBINED WITH ARTERIAL HYPERTENSION**

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Significant progress in rheumatoid arthritis pharmacotherapy is associated with the implementation of synthetic-derived immunosuppressors and genetically engineered (biological) drugs into clinical practice. Arterial hypertension, like rheumatoid arthritis, is accompanied by producing a large amount of inflammatory cytokines, namely TNF- α . The purpose of the work was to study the cardiotropic effects of leflunomide and etanercept against the background of experimental rheumatoid arthritis associated with arterial hypertension in rats. Experiments on mature adult, non-linear white rats found that leflunomide and etanercept did not affect the degree of hypertension against the background of adjuvant arthritis, but manifested an antihypertensive effect when used in adjuvant arthritis combined with arterial hypertension. Leflunomide leads to an increase in heart rate by 5–10.4% at different terms of observation, both against the background of adjuvant arthritis only and under the combined pathology conditions. Etanercept, when used against adjuvant arthritis, causes bradycardia but prevents the development of tachycardia, which is detected in untreated animals with a comorbid condition during manifestation and attenuation of the inflammatory process. The study findings may be relevant for development of new approaches to the treatment of rheumatic and cardiac pathology.

Keywords: leflunomide, etanercept, cardiotropic action, rheumatoid arthritis, hypertension, comorbid pathology

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Pathogenetic aspects of the onset and development of rheumatoid arthritis (RA) substantiate the pathogenetic basis of pharmacotherapy and determine the feasibility of using drugs of appropriate pharmacotherapeutic groups. Significant progress has been made in the pharmacotherapy of RA over the past decades, which is due to implementation of synthetic-derived immunosuppressants and genetically engineered (biological) drugs (GEBD) into clinical practice [2, 5].

The fundamental role in the pathogenesis of RA is played by TNF α , a pleiotropic cytokine with anti-inflammatory and immunomodulatory activity [3]. Among the GEBP, TNF- α inhibitors were the first GEBP implemented into practice, and today they are ranked among this class drugs as "the first-line" drugs [2].

It is TNF- α that is one of the major cytokines to determine the development of synovial inflammation and osteoblast-mediated bone destruction in arthritis. Therefore, TNF- α is one of the main pharmacological targets for anticytokine therapy of RA and other inflammatory joint diseases.

The issues of the proinflammatory cytokines role in the origin and development of cardiac pathology are highlighted and discussed in the scientific periodicals [7, 8]. It is known that due to heart failure, the heart produces a large amount of inflammatory cytokine, namely TNF- α , whose content and activity correlate with the degree of the left ventricle function impairment, the presence of changes in the myocardium, accompanied by a decrease in contractile function, can lead to apoptosis of cardiomyocytes with the direct link of this cytokine with the corresponding receptors on cardiomyocytes [3, 11].

These facts have caused the prediction that lowering the level of TNF- α may have a positive effect on the functional status of the heart in the conditions of the heart failure and clinical manifestations of cardiac pathology. Experimental data and results of the limited clinical trial confirmed this result, however, the results of the large-scale controlled trials have shown a negative result and, in particular, worsening of state in patients with heart failure with the use of etanercept or infliximab [3].

Particularly acute is the issue of the safety and efficacy with the use of genetically modified and synthetic immunosuppressors against the background of combined pathology, in particular against the background of RA associated with hypertension. The side, namely, cardiotoxic effects of synthetic and genetically engineered immunosuppressants have not yet been sufficiently studied [7, 8].

Immunosuppressors can exhibit both general toxic effects [1] and cardiotoxicities, including the ability to increase the risk of hypertension, destabilization of blood pressure, increased preexisting hypertension, heart failure, heart rhythm disorders, etc. [3, 11]. There is no consensus among the world

researchers about the cardiac safety and cardio-efficacy of synthetic and biological immunosuppressors. For example, leflunomide (LF) in various RA patients may cause either hyper- or hypotension [5]. The effect of immunosuppressors on the cardiovascular system's condition against the background of RA combined with hypertension has not been studied [6, 12]. It is precisely because of the common pathogenesis link regarding the increase in the content and activity of proinflammatory cytokines in cardiac diseases associated with RA, namely, arterial hypertension (AH), that there is a prediction about the possibility of using immunosuppressors against the background of comorbid pathology of inflammatory nature.

Particular interest is drawn to the study on the efficacy of biological immunosuppressors that specifically block the activity of TNF- α , against the background of both RA and comorbid cardiac pathology, in particular AH.

Quite effective in its implementation into clinical practice was the drug – TNF- α inhibitor – Etanercept (ENB), which binds to biologically active TNF- α (blocks its activity and prevents the latter from binding to the corresponding receptors). The drug regulates the activity of TNF- α in RA, including – juvenile, psoriatic arthritis, psoriasis, ankylosing spondylitis [2].

The purpose of the work was to study the cardiotropic effect of leflunomide and etanercept against the background of experimental rheumatoid arthritis associated with hypertension in rats.

Materials and methods. The experiments were performed on mature non-linear white rats of both sexes with a starting weight of 168.5 ± 3.42 g, which were kept on a standard balanced diet in vivarium under free access to food and water at the temperature of 20–22° C and relative humidity of 40–60%. The studies were carried out in compliance with the requirements of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986). After acclimatization (14 days) under normal diet and with free access to food and water in the laboratory room in special plastic cages of 7–8 specimens of similar sex in each random sample, white rats were divided into groups.

The first group included 20 intact animals that were kept in the laboratory room under the similar conditions and with the same duration as the animals of other experimental groups. The second group included white rats who were administered a complete Freund's adjuvant (AF) to simulate experimental RA (adjuvant arthritis – AA). The animals of the third group consisted of rats, which were induced AH. The animals of the fourth group were simulated a comorbid state – AH + AA. Animals of the fifth and sixth groups started treatment with LF and ENB 7 days after AF administration against the background of the formed AH. The seventh and eighth groups included rats with a comorbid condition, whose treatment with LF and ENB drugs, respectively, also began 7 days after the AF administration against the background of already formed hypertension. Therefore, animals that were only simulated AA (AA control), as well as AA rats (monopathology) treated with LF and ENB were not subject to salt loading at all. Simulation of hypertension was performed by means of salt loading, which was created by providing the animals with salt drinking (replacement of drinking water with saline solution – 1% sodium chloride solution) under the conditions of free access to it. Duration of hypertension development was 21 days [1, 9, 13]. Experimental RA was induced by subcutaneous injection of complete AF into the plantar part of the hind limb (left), which, according to the literature, is one of the most adequate agents capable of inducing the said pathological condition. The mechanism of developing AA induced by administration of complete AF, clinical symptoms, as well as the effects of drugs are most similar to those observed in humans with RA.

Comorbid pathology was simulated as follows: against the background of hypertension (on the 21st day from the start of AH model formation), the animals were singly administered complete AF. All animals were measured baseline BP and cardiac rate (CR) by a sphygmomanometric method with the Ugo Basile device (Italy). In rats subjected to salt loading, blood pressure was measured after 21 days. Animals that were not recorded an increase in blood pressure after 21 days from the beginning of the salt loading were not subject to the distribution into groups and were removed from the experiment under the relevant rules of euthanasia.

After randomization of rats with AH, the corresponding groups of animals were formed, each including almost the same number of rats of different sexes. LF was used as follows: the first three days at the dose of 15 mg/kg (shock dose), and then – daily at the dose of 1.5 mg/kg (therapeutic dose). The drug was injected through a special metal probe into the stomach in 1% starch suspension once a day (at 10 to 11 o'clock). Treatment of the animals was started 7 days after the AF administration (acute period of proper

AA). Duration of the animals' observation was 12 weeks from the start of the AH simulation, of which 21 days (3 weeks) was the formation of AH, then – 9 weeks after the AF administration against the background of the formed AH (including 8 weeks of treatment, which began 7 days after the AF administration). Administration of ENB was carried out subcutaneously at the dose of 4 mg/kg, which actually corresponds to the therapeutic dose for humans in terms of animals (rats). Administration of ENB was performed four times (once a week, in the same dose) – 7 days, 14 days, 21 days and 28 days after the complete AF administration.

Data Processing. The normality of distribution was assessed by the Shapiro–Wilk (W) criterion. Data are presented as the arithmetic mean and the standard error of the mean representativeness. The probability between the means in the two samples was determined using the Student test with normal distribution. Differences were considered statistically significant at a significance level of at least 0.05.

Results of the study and their discussion. No significant fluctuations in blood pressure in the animals of the intact group were observed. Experimental RA was accompanied by the development of hypertension, starting from the first days after the AF administration, which was characterized by an increase in blood pressure by 11–12% (table 1).

In animals with the salt load (third group) there was an increase in blood pressure by 17.9–27.0 % on the 7th–21st day of observation. It should be noted that with the increase in the term of salt load up to the 21st day the number of animals with an increase in blood pressure increased. Thus, 7 days after the AH model formation, 58.8% of white rats increased their blood pressure by 19%; blood pressure increased by 27% in 82.4% of animals on the 14th day of AH formation. The highest number of animals – 88.2% with elevated blood pressure by 17.9% was recorded on the 21st day.

It is this term that characterizes the actual presence of hypertension (the AH model) in white rats, as noted above. A significant increase in blood pressure in animals with salt loading (rats of the third group) was observed throughout the period of animal observation, however, starting from the 42nd–45th day from the start of daily use of 1% sodium chloride solution as a drink (free access of animals to drinking), a decrease in the degree of hypertension was recorded compared to the value registered on the 21st day.

Under the conditions of comorbid pathology modeling (administration of AF against the background of formed AH), there was no further increase of arterial pressure (AP) compared to the data recorded in animals with AH. Thus, complete AF does not lead to an increase in blood pressure against the background of the formed hypertension (i.e., AF does not lead to an increase in hypertension against the background of already developed disease; table 1). At the same time, during the acute period of the inflammatory process development and the period of its generalization (the 28th day after the AF administration), blood pressure remained significantly higher against the background of hypertension than that in intact animals, as well as in animals with AA alone.

According to the data in table. 1, LF did not lead to a decrease (normalization) of AP in AA rats, and throughout its duration, AP values remained similar to those observed in untreated AA animals (increased by more than 10%). The use of LF against the background of comorbid pathology, on the contrary, led to a significant decrease in blood pressure, the most significant during the period of AA decrement (the value of blood pressure in animals treated with LF decreased by 10% compared to the value in animals with a comorbid condition and reduced by 19.7 % compared to the value on the 21st day from the salt loading beginning).

Thus, the use of LF should be associated with continuous monitoring of blood pressure. In our opinion, against the background of AA alone, the use of LF should be combined with antihypertensive agents, which can only be confirmed or denied by performing a clinical trial.

With the use of ENB against AA, no significant increase in blood pressure was observed in rats, however, a rise in blood pressure of 4–8 % compared to baseline values was observed in this group (table 1). Thus, ENB did not lead to aggravated hypertension in the experimental RA. Under the conditions of comorbid pathology, ENB did not cause hypertension, but, on the contrary, significantly reduced blood pressure in rats during AA manifestation against the background of hypertension (recorded on the 21st day from the beginning of the salt loading) and reduced this index by 17% compared to values in untreated animals during the period of AA decrement (on the 60th day after AF administration). At the same time, blood pressure during the inflammation generalizing period remained higher than that of intact animals.

Blood pressure ($x \pm SE$, mm Hg) in rats using Leflunomide and Etanercept against adjuvant arthritis combined with arterial hypertension

Group, index	Term of observation, day ^Z					
	Initial data [•]	7 (21+7)	14 (21+14)	28 (21+28)	42-45 (21+42)	56-60 (21+60)
Intact animals, n	92.2±4.2 20	93.4±1.4 20	90.7±2.8 20	92.2±1.4 20	93.2±1.8 20	94.7±1.2 20
AA n, p (up to output values of the given group)	87.4±1.54 40	97.5±1.52 40 ≤0.05	97.9±1.70 40 ≤0.05	97.5±1.52 40 ≤0.05	97.2±1.50 40 ≤0.05	97.2±2.0 40 ≤0.05
AH (initial value – the 21st day), n	111.2±1.9* 33	107.0±1.4* 33	111.7±1.4* 33	108.2±2.5 [#] 33	101.6±2.0* [#] 33	101.6±2.0* [#] 33
AH+AA n	115.9±5.3* 25	106.4±2.8* 25	103.6±4.6* 25	100.2±3.7* 25	99.1±1.60 25	97.2±4.1 25
AA+LF n, p (up to output values of the given group)	87.4±1.16 15	97.4±3.35 15 ≤0.05	97.7±3.75 15 ≤0.05	95.7±2.37 15 ≤0.05	96.0±2.90 15 ≤0.05	98.1±4.0 15 ≤0.05
AA+ENB n, p (up to output values of the given group)	89.0±3.7 15	99.9±2.24 15 ≤0.05	96.4±3.7 15 ≥0.05	95.0±1.9 15 ≥0.05	92.7±3.4 15 ≥0.05	96.8±3.7 15 ≥0.05
AH+AA+ +LF, n	109.4±2.4* 15	106.4±2.8* 15	99.8±4.02 [#] 15	93.0±2.3 ^{#Δ} 15	96.0±4.85 15	87.8±1.7* ^{#Δσ} 15
AH+AA+ +ENB, n	115.9±5.3* 15	99.8±2.3 ^{#Δ} 15	96.4±4.3 [#] 15	104.2±4.3* ^{#α} 15	102.3±3.0* [#] 15	96.0±2.5 ^{#α} 15

Notes (here and in the following table): 1. * – $p \leq 0.05$ - the index difference in the given observation period compared to its value in intact animals. 2. # – $p \leq 0.05$ the index difference in the given observation period compared to the value in animals with hypertension on the 21st day. 3. Δ – $p \leq 0.05$ the index difference in the given observation period compared to the value in animals with hypertension for the same period of observation. 4. σ – $p \leq 0.05$ the index difference in the given observation period compared to the value in animals with combined pathology (AH + AA). 5. • – for the initial data, the values of the studied parameters, which were registered in animals on the 21st day from the beginning of the salt load, i. e., the value of blood pressure in animals with formed AH, were taken. 6. Z – the observation period is specified after the AF administration against the background of AH, in parentheses – the term of AH formation + the term starting from the AF administration).

Thus, the results of the studies showed that neither LF nor ENB enhanced hypertension caused by AF, and blood pressure during the action of these drugs remained elevated, similarly to the values in untreated animals against AA. Against the background of experimental RA in rats there was a significant increase in heart rate up to the 42nd–45th day after AF administration (table 2).

Statistically significant changes (decrease) in the cardiac rate should be noted 60 days after the induction of the inflammatory process. At the same time, the animals with formed AH against the background of continued saline solution consumption for more than 21 days showed a decrease in heart rate in the period from the 28th to the 81st day of observation (from the beginning of the salt loading), on average – by 12%. Thus, under the conditions of prolonged salt loading (more than 21 days), bradycardia develops in animals.

In the acute period of AA development against the background of AH and, particularly, in the period of AA decrement – on the 56th–60th days – significant changes in the cardiac rate (by 28.6%) were recorded compared to this index in animals with monopatology (AH), which were defined as tachyarrhythmia, and on the 42nd day of observation, they were also reliable compared to those observed in intact animals and testified to the 13.5% increase in the cardiac rate (table. 2). Significant changes (increase) of the cardiac rate by 5–10.4 % were caused by the synthetic immunosuppressor LF in different terms of its application against the background of AA.

The use of LF against the background of a comorbid condition was also accompanied by a significant (compared to the value in intact animals and rats with hypertension) increase in the cardiac rate, at the same time, the indicated reaction in this group was slightly weaker than that observed with the use of LF only against the background of AA. This fact can be regarded as a warning when applying LF both against the background of monopatology (AA) and in the comorbid state (AH + AA) and indicate the need to monitor the frequency and rhythm of heart rate and the use of appropriate therapies.

With the use of the ENB against the background of AA alone, a significant decrease in the cardiac rate was recorded (table 2), starting from the 28th day after AF administration and until the end of the observation period (the 60th day after AF administration) by 9.2–11.2 % compared to data recorded in animals with comorbid condition and intact rats.

Cardiac rate ($x \pm SE$, beats/min) in rats using Leflunomide and Etanercept against the background of experimental rheumatoid arthritis associated with hypertension

Group, index	Term of observation, day ^Z					
	Initial data [•]	7 (21+7)	14 (21+14)	28 (21+28)	42-45 (21+42)	56-60 (21+60)
Intact animals, n	368.3±3.3 20	370.7±4.3 20	365.1±11.4 20	368.4±2.1 20	363.3±10.7 20	372.3±11.2 20
AA n	365.2±8.8 40	398.9±8.1* 40	396.7±8.7* 40	386.8±7.2* 40	354.1±6.4 40	340.1±9.8*# ⁶ 40
AH n	365.1±12.4 33	326.3±2.4*# 33	321.5±5.3*# 33	326.3±2.4*# 33	325.7±9.8*# 33	328.7±10.2*# 33
AH+AA n	378.6±9.2 25	383.7±9.6 ^Δ 25	371.4±3.5* ^Δ 25	367.1±9.2 ^Δ 25	385.0±3.5 ^Δ 25	422.6±10.2*# ^Δ 25
AA+LF n	378.6±4.76 15	-	398.5±7.8*# ^{Δ6} 15	417.9 ±17.7*# ^{Δ6} 15	417.8±6.3*# ^{Δ6} 15	416.8±8.2*# ^Δ 15
AA+ENB n, p (up to output values of the given group)	361.1±6.2 15	363.0±8.4 15 ≥0.05	363.9±6.2 15 ≥0.05	326.7±7.4* ⁶ 15 ≤0.05	320.6±9.4* ⁶ 15 ≤0.05	326.±4.9* ⁶ 15 ≤0.05
AH+AA+LF n	378.6±4.76 15	-	390.5±6.8* ^{Δ6} 15	380.0±5.8 ^Δ 15	390.0±9.7 ^Δ 15	392.9±3.9* ^Δ 15
AH+AA+ENB, n	369.3±5.6 15	378.3±9.2 ^Δ 15	354.2±8.7 ^Δ 15	366.1±7.4 ^Δ 15	347.8±6.2* ⁶ 15	342.9±9.9* ⁶ 15

In general, our results are consistent with those obtained by other researchers [3, 8, 11, 13]. ENB, immunosuppressant of biological origin, prevented the development of tachycardia, which was recorded in untreated animals with a comorbid condition during the manifestation and decrement of the pathological process [3]. At the same time, a significant decrease in the cardiac rate on the 42nd-60th day of observation compared to values in intact animals and in rats with a comorbid condition was reported with the use of ENB [4, 11]. Ability of LF and ENB to influence the rhythm and the cardiac rate of rats against the background of AH associated with AA indicates the feasibility of the rhythm and cardiac rate monitoring with the use of immunosuppressors [10, 13].

Conclusions

1. Leflunomide and etanercept do not affect the degree of hypertension against adjuvant arthritis, which is confirmed by a significant increase of blood pressure in rats (by more than 10%) similar to that observed in untreated animals with inflammatory process.
2. Leflunomide and etanercept have an antihypertensive effect when used against adjuvant arthritis combined with arterial hypertension, as evidenced by a decrease in the hypertension degree by 9–20 % and 17%, respectively, at different terms of inflammation development in rats compared to values in untreated animals with a comorbid condition.
3. White rats against the background of arterial hypertension combined with AA develop tachyarrhythmia, which is characterized by a significant increase in the cardiac rate by 28.6% compared to this index in animals with monopathology – arterial hypertension.
4. Leflunomide leads to an increase in the cardiac rate by 5–10.4 % at different terms of observation, both against the background of adjuvant arthritis only and under conditions of combined pathology. Etanercept, when used against adjuvant arthritis, causes bradycardia, but prevents the development of tachycardia, which is detected in untreated animals with a comorbid condition during the manifestation and attenuation of the inflammatory process.

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Реферати

**КАРДИОТРОПНИЙ ВПЛИВ ІМУНОСУПРЕСОРИВ
СИНТЕТИЧНОГО І ГЕННО-ІНЖЕНЕРНОГО
ПОХОДЖЕННЯ У ЩУРІВ
ЗА ЕКСПЕРИМЕНТАЛЬНОГО РЕВМАТОЇДНОГО
АРТРИТУ, ПОЄДНАНОГО З АРТЕРІАЛЬНОЮ
ГІПЕРТЕНЗІЄЮ**

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Істотний прогрес у фармакотерапії ревматоїдного артриту пов'язаний з впровадженням в клінічну практику імуносупресорів синтетичного походження і генно-інженерних (біологічних) препаратів. Артеріальна гіпертензія, як і РА, супроводжується продукцією великої кількості запального цитокіну, а саме - ФНП- α . Метою дослідження було вивчення кардіотропного впливу лефлуноміда і етанерцепта на тлі експериментального ревматоїдного артриту, асоційованого з артеріальною гіпертензією, у щурів. У досліджах на статевозрілих нелінійних білих щурах встановлено, що лефлуномід і етанерцепт не впливають на ступінь гіпертензії на тлі ад'ювантного артриту, але виявляють антигіпертензивний ефект при застосуванні на тлі ад'ювантного артриту, поєднаного з артеріальною гіпертензією. Лефлуномід призводить до зростання частоти серцевих скорочень на (5-10,4)% в різні терміни спостереження як на тлі лише ад'ювантного артриту, так і в умовах поєднаної патології. Етанерцепт при застосуванні на тлі ад'ювантного артриту викликає брадикардію, але запобігає розвитку тахікардії, що реєструється у нелікованих тварин з коморбідним станом в період маніфестації і згасання запального процесу. Результати досліджень можуть мати значення для розробки нових підходів в лікуванні ревматичної та кардіальної патології.

Ключові слова: лефлуномід, етанерцепт, кардіотропна дія, ревматоїдний артрит, артеріальна гіпертензія, коморбідна патологія.

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**КАРДИОТРОПНОЕ ВЛИЯНИЕ
ИМУНОСУПРЕССОРОВ СИНТЕТИЧЕСКОГО
И ГЕННО-ИНЖЕНЕРНОГО ПРОИСХОЖДЕНИЯ
У КРЫС ПРИ ЭКСПЕРИМЕНТАЛЬНОМ
РЕВМАТОИДНОМ АРТРИТЕ, ОБЪЕДИНЕННОМ
С АРТЕРИАЛЬНОЙ ГИПЕРТЕНЗИЕЙ**

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Существенный прогресс в фармакотерапии ревматоидного артрита связан с внедрением в клиническую практику иммуносупрессоров синтетического происхождения и генно-инженерных (биологических) препаратов. Артериальная гипертензия, как и РА, сопровождается продукцией большого количества воспалительного цитокина, а именно – ФНО- α . Целью исследования было изучение кардиотропного влияния лефлуномида и этанерцепта на фоне экспериментального ревматоидного артрита, ассоциированного с артериальной гипертензией, у крыс. В опытах на половозрелых неллинейных белых крысах установлено, что лефлуномид и этанерцепт не влияют на степень гипертензии на фоне адьювантного артрита, но проявляют антигипертензивный эффект при применении на фоне адьювантного артрита, объединенного с артериальной гипертензией. Лефлуномид приводит к росту частоты сердечных сокращений на (5-10,4)% в разные сроки наблюдения как на фоне только адьювантного артрита, так и в условиях сочетанной патологии. Этанерцепт при применении на фоне адьювантного артрита вызывает брадикардию, но предотвращает развитие тахикардии, что регистрируется у нелеченных животных с коморбидным состоянием в период манифестации и угасания воспалительного процесса. Результаты исследований могут иметь значение для разработки новых подходов в лечении ревматической и кардиальной патологии.

Ключевые слова: лефлуномид, этанерцепт, кардиотропное действие, ревматоидный артрит, артериальная гипертензия, коморбидная патология.

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ROLE OF NITRIC OXIDE IN DEVELOPMENT OF FIBROTIC CHANGES IN RATS' TESTES AFTER 270 DAY CENTRAL DEPRIVATION OF TESTOSTERONE SYNTHESIS

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Disturbance in production of nitric oxide (NO) may lead to various changes in different organs and systems. Certain clinical situations require prolonged usage of inhibitors of testosterone synthesis. Scientific literature provides limited information regarding the influence of prolonged deprivation of testosterone synthesis on production of NO and microscopic organization of rats' testes. Prolonged central deprivation of testosterone synthesis leads to endothelial dysfunction, development of fibrosis, decreases the nitric oxide production and shifts pro-/antioxidant balance in favor of the pro-oxidants without increase in lipid peroxidation intensity. Central deprivation of testosterone synthesis leads to fibrosis with subsequent disruption of the structural organization of the convoluted seminiferous tubules, hemodynamic disturbances, endothelial dysfunction, increased density of the vascular wall of blood vessels and systemic stasis. Decreased production of NO from constitutive isoforms of NO-synthase plays major role in development of structural changes in the interstitial tissue of testes on the 270th day of the experiment.

Keywords: testes; rats; diphereline; oxidative stress; nitric oxide; fibrosis.

The study is a fragment of the research project "Experimental morphological study of cryopreserved placenta transplants action diphereline, ethanol and 1% methacrylic acid on the morphofunctional status in a number of internal organs", state registration No. 0119U102925.

Disturbance in production of nitric oxide (NO) may lead to various changes in different organs and systems. There is evidence that NO produced by inducible isoform of NO-synthase (EC 1.14.13.39, iNOS) may lead to liver fibrosis through increase in peroxynitrite (ONOO⁻) formation [1]. Liver is not the only organ in which excessive iNOS activity leads to fibrotic changes [2, 4].

Increased iNOS activity in rat testes during diabetes also leads to fibrosis and increased lipid peroxidation [12]. At the same time, decrease in endothelial NOS activity and testosterone content may also lead to disruption of erectile function [8]. Therefore, changes in quantity and source of NO production are important to testicular tissue metabolism.

Certain clinical situations require prolonged usage of testosterone synthesis inhibitors [7]. Testosterone metabolism is closely related to endothelial NOS activity, which is especially true during testosterone deficiency [5]. Testosterone synthesis is necessary for suppression of reactive nitrogen (RNS) and oxygen species (ROS) formation [10]. A decrease in testosterone synthesis, caused by intervention in autoregulation of its production by interstitial endocrinocytes, leads to decrease of nitric oxide formation from constitutive isoforms of NOS with simultaneous elevation of its production by iNOS [10]. This change in the source of NO production in turn causes two adverse events. First one is the uncoupling of constitutional NOS isoforms, which leads to increased production of ROS from microsomal electron transport chains. Second event is overproduction of NO by iNOS. The last event has two major consequences: increased nitrite accumulation (as the result of excessive NO oxidation by oxygen present in tissues) and peroxynitrite formation (in reaction between NO and superoxide anion radical). Therefore deficiency of testosterone synthesis may cause the development of nitritive and nitrosative stress, in addition to the oxidative stress.

Scientific literature provides limited information regarding the influence of prolonged deprivation of testosterone synthesis on production of NO and microscopic organization of rats' testes.

The purpose of the study was to determine the microscopic organization of rats' testes, to determine the sources of nitric oxide production and the intensity of oxidative stress in the rats' testes during experimental central deprivation of testosterone synthesis by diphereline injection on the 270th day of the experiment.

Materials and methods. The experiments were carried out on 10 sexually mature male white rats of the Wistar line. Rats were divided into 2 groups: the control group (5) and the experimental group (5). Animals from the experimental group were injected subcutaneously with diphereline (Triptorelin embonate) at a dose of 0.3 mg of the active substance/ per kg of body weight for 270 days, while the control group received injection of saline [3]. Animals were kept in standard vivarium conditions of the Ukrainian Medical Stomatological Academy. Experimental animals were sacrificed in strict compliance with the provisions of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes"; (Strasbourg, 1986), as well as with the "General Ethical Principles of Animal Experiments" adopted by the First National Congress on Bioethics (Kyiv, 2001).

After an overdose of ketamine, the animals were decapitated, the prepared small pieces of the testes were fixed in a 2.5% glutaraldehyde solution (pH=7.2-7.4). Postfixation of the material was carried out with 1% solution of osmium (IV) oxide, followed by dehydration in propylene oxide and a sample was embedded into the epoxy resins mixture. Ultrathin sections made with an ultramicrotome were contrasted with a 1% aqueous solution of uranyl acetate and lead citrate according to the Reynolds' method and studied with an electron microscope [1].

Using standard methods, the material was imbedded in paraffin blocks, of which sections 4 μ m thick were made and stained with hematoxylin and eosin. Histological preparations were examined using Biorex 3 light microscope with digital microfilter with software adapted for these studies (Serial No. 5604).

All biochemical studies were carried out in 10% homogenate of testis tissue using Ulab 101 spectrophotometer. General activity of NO-synthase (gNOS), activity of constitutive isoforms (cNOS), activity of inducible isoform (iNOS), activity of arginases and nitrite concentration was determined by methods described by Yelinska A.M. [11].

Basic production of superoxide anion radical ($O_2^{\cdot-}$), its production by the mitochondrial electron transport chain (ETC) and microsomal ETC was determined by the growth of diformazan concentration, formed in the reaction of $O_2^{\cdot-}$ with nitro blue tetrazolium [11]. Superoxide dismutase (SOD) activity was determined by inhibition of adrenaline autooxidation, while catalase activity was determined by the amount of hydrogen peroxide, remained after its catalase-dependent reduction [11]. The concentration of free malondialdehyde (MDA) was determined by reaction with 1-methyl-2-phenylindole.

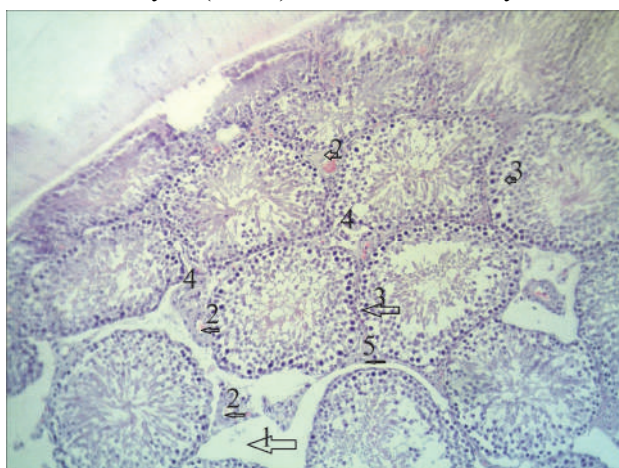


Fig. 1.a. Seminiferous tubules of experimental rat on the 270th day. Microimage. Stain: hematoxiline and eosine. Lens: 10; Ocular lens: 10. 1. Interstitial space - fibrosis. 2. Blood vessel 3. Spermatogenic epithelium of the tubule. 4. Interstitial cells. 5. The capillary in the interstitium.

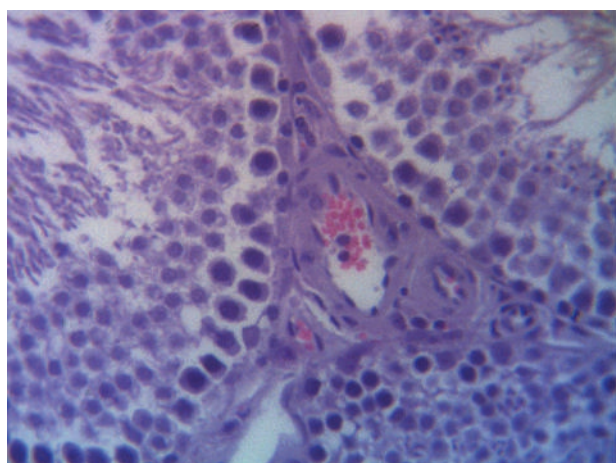


Fig. 1.b. Interstitial space of experimental rat on the 270th day. Microimage. Stain: hematoxiline and eosine. Lens: 40; Ocular lens: 15.

From the side of interstitial endocrinocytes, there is a tendency to a quantitative decrease in comparison with the control group. Also, there are interstitial spaces between the convoluted tubules with a complete absence of interstitial endocrinocytes. The cells themselves are reduced in size, the nuclei are heterochromic, in the cytoplasm there is a small amount of lipid granules (fig. 1c).

Statistical processing of the study results was carried out using the Microsoft Office Excel software and the Real Statistics 2019 extension to it. The nonparametric Mann-Whitney test was used to determine the statistical significance of differences between the groups. The difference was considered statistically significant at $p < 0.05$.

Results of the study and their discussion. When studying semi-thin sections of the testes on the 270th day of the experiment, we found changes in the interstitial tissue, which are very characteristic of fibrosis (fig.1a). We also observed violation of the structural organization of the convoluted seminiferous tubules. Disturbances in the microvasculature manifested as endothelial dysfunction and increase in density of the vascular wall.

Rats from the experimental group had an increase in connective tissue spaces associated with both the qualitative and quantitative composition of the altered interstitial cells and the microvasculature. Structural reorganization of the interstitial tissue manifested itself as a quantitative increase in arterioles, venules, and capillaries. We observed morphological signs of endothelial dysfunction, hemodynamic disturbances, perivascular fibrosis with a decrease in the volume of the microvascular bed with subsequent fibrosis of the interstitium in general. We determined vasodilation of arterioles and venules, tortuosity of precapillaries in the visual field. Capillaries were enlarged against the background of general stasis (fig. 1b).

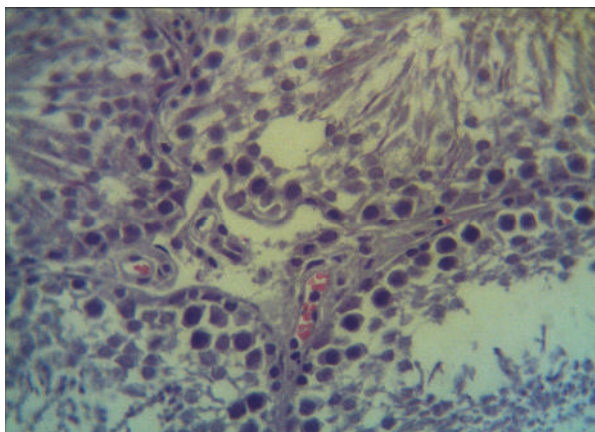


Fig. 1.c. Interstitial space of experimental rat on the 180th day. Microimage. Stain: hematoxyline and eosine. Lens: 40: Ocular lens:10.

by 46.52%, while activity of catalase is was also decreased by 40.33%. There were no statistically significant changes in concentration of free MDA

Table 1

Oxidative stress markers in rats' testes during 270-day central testosterone synthesis deprivation (M±m)

Groups	Parameters					
	SOD activity, c.u.	Catalase activity, nkat/g of tissue	Basic O ₂ ⁻ production, nmol/s per g of tissue	Production of O ₂ ⁻ from mitochondrial ETC, nmol/s per g of tissue	Production of O ₂ ⁻ from microsomal ETC, nmol/s per g of tissue	Free MDA, μmol/g of tissue
Control	1.87±0.11	182.0±17.0	0.26±0.01	7.84±0.13	9.55±0.19	6.64±1.44
Experimental	1.00±0.19*	108.6±9.3*	1.30±0.02*	12.85±0.13*	11.71±0.11*	9.15±0.26

Note: * - indicates that the difference is statistically significant when compared with control group (p<0.05)

On the 270th day of the experiment we detected a decrease in gNOS activity by 68.51% (tab. 2). There were no statistically significant changes in activity of iNOS in rats' testes after 270 days of central deprivation of testosterone synthesis. Activity of cNOS isoforms dropped by 8.2 times. Arginase activity decreased by 39.92%. Concentration of nitrites in testes lowered by 25.33%.

Table 2

Nitric oxide cycle function during 270-day central testosterone synthesis deprivation (M±m)

Groups	Parameters				
	gNOS activity, μmol/min per g of protein	iNOS activity, μmol/min per g of protein	cNOS activity, μmol/min per g of protein	Arginase activity, μmol/min per g of protein	NO ₂ ⁻ concentration, nmol/L
Control	0.54±0.04	0.13±0.02	0.41±0.03	2.48±0.05	3.83±0.25
Experimental	0.17±0.02*	0.13±0.02	0.05±0.0003*	1.49±0.11*	2.86±0.16*

Note: * - indicates that the difference is statistically significant when compared with control group (p<0.05)

Nitric oxide is synthesized in lower quantity and predominantly from inducible isoform of NO-synthase. Despite increased production of pro-oxidants and decreased activity of antioxidant enzymes, the intensity of lipid peroxidation is not elevated.

The reason for decreased production of NO is lowered activity of cNOS since activity of iNOS is within the values of the control group. This situation is the direct result of testosterone deficiency caused by experimental procedure. Also lowered activity of cNOS may contribute to the endothelial dysfunction observed in fig. 1b.

Lowered concentration of nitrites in rats' testes during prolonged central deprivation of testosterone synthesis can be the result of the increased activity of nitrite reductases, which is aimed at compensation of NO production deficiency.

In our previous study we observed increased iNOS activity and decreased activity of arginase [9]. Since iNOS and arginase can be considered as "marker enzymes" for definition of macrophage polarization, we can consider that most of the macrophages present in testes on the previous term of experiment were in M1 (proinflammatory) polarization [9].

The prevalence of parietal macrophages over the interstitial ones still remains on the 270th day of the experiment. However, we discovered that arginase activity is much greater than iNOS activity in this

term of experiment. This fact gives us grounds to speculate that a major part of macrophages, present in testes on the 270th day of experiment, have M2 (anti-inflammatory) polarization.

We observed a peculiar situation, when superoxide anion-radical production is increased, while SOD and catalase activities are decreased, however lipid peroxidation is not increased. Since free MDA levels were not changed after 270-day central deprivation of testosterone synthesis we can conclude that increased superoxide anion-radical is used for remodeling of the tissue and it does not cause cell damage. Without oxidative damage to the cell membrane SOD and catalase genes are not expressed hence the lowered activity of these enzymes. But we must also consider the possible influence of other antioxidants like glutathione system and non-enzymatic antioxidants.

Decreased cNOS activity may be the key factor contributing to increased production of superoxide anion from mitochondrial ETC since NO produced from neuronal isoform of NOS located in mitochondria has ability to regulate superoxide anion-radical production [13].

Conclusions

Central deprivation of testosterone synthesis leads to fibrosis with subsequent disruption of the structural organization of the convoluted seminiferous tubules, hemodynamic disturbances, endothelial dysfunction, increased density of the vascular wall of blood vessels and systemic stasis. Decreased production of NO from constitutive isoforms of NO-synthase plays a major role in development of structural changes in the interstitial tissue of testes on the 270th day of the experiment.

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Реферати

РОЛЬ ОКСИДУ АЗОТУ В РОЗВИТКУ ФІБРОТИЧНИХ ЗМІН СІМ'ЯНИКІВ ЩУРІВ ПІСЛЯ 270 ДНІВ ЦЕНТРАЛЬНОЇ ДЕПРИВАЦІЇ СИНТЕЗУ ТЕСТОСТЕРОНА

Стецук Є.В., Акімов О.Є., Шепітько К.В.,
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Порушення виробництва оксиду азоту (NO) може призводити до різних змін в різних органах і системах. Певні клінічні ситуації вимагають тривалого використання інгібіторів синтезу тестостерону. У науковій літературі є обмежена інформація про вплив тривалого позбавлення синтезу тестостерону на продукцію NO і мікроскопічну організацію сім'яників щурів. Тривала центральна депривація синтезу

РОЛЬ ОКСИДА АЗОТА В РАЗВИТИИ ФИБРОТИЧЕСКИХ ИЗМЕНЕНИЙ В ТЕСТАХ КРЫС ПОСЛЕ 270 ДНЕЙ ЦЕНТРАЛЬНОЙ ДЕПРИВАЦИИ СИНТЕЗА ТЕСТОСТЕРОНА

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Нарушение производства оксида азота (NO) может приводить к различным изменениям в разных органах и системах. Определенные клинические ситуации требуют длительного использования ингибиторов синтеза тестостерона. В научной литературе имеется ограниченная информация о влиянии длительного лишения синтеза тестостерона на продукцию NO и микроскопическую организацию семенников крыс. Длительная центральная

тестостерону призводить до ендотеліальної дисфункції, розвитку фіброзу, знижує вироблення оксиду азоту та зрушку про- / антиоксидантний баланс на користь прооксидантів без збільшення інтенсивності перекисного окислення ліпідів. Центральна депривація синтезу тестостерону призводить до фіброзу з подальшим порушенням структурної організації звивистих сім'яних каналців, порушень гемодинаміки, ендотеліальної дисфункції, збільшення щільності судинної стінки кровоносних судин і системному застою. Зниження продукції NO з конститутивних ізоформ NO-синтази відіграє основну роль у розвитку структурних змін інтерстиціальної тканини сім'яників на 270-й день експерименту.

Ключові слова: сім'яники, інтерстиціальні ендокриноцити, суспендоцити, NO-синтаза, iNOS, cNOS, L-аргінин, супероксиддисмутаза, щури.

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депривація синтезу тестостерону приводить к ендотеліальної дисфункції, розвитку фіброза, знижує вироботку оксида азота і сдвигает про- / антиоксидантний баланс в пользу прооксидантов без увеличения интенсивности перекисного окисления липидов. Центральная депривация синтеза тестостерона приводит к фиброзу с последующим нарушением структурной организации извитых семенных канальцев, нарушениям гемодинамики, эндотелиальной дисфункции, увеличению плотности сосудистой стенки кровеносных сосудов и системному застою. Снижение продукции NO из конститутивных изоформ NO-синтазы играет основную роль в развитии структурных изменений интерстициальной ткани семенников на 270-й день эксперимента.

Ключевые слова: семенники, интерстициальные эндокриноциты, суспендоциты, NO-синтаза, iNOS, cNOS, L-аргинин, супероксиддисмутаза, крысы.

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MORPHOMETRIC ASSESSMENT OF STRUCTURAL CHANGES IN THE DUODENAL WALL OF RATS CAUSED BY SKIN BURN INJURY UNDER CONDITIONS OF EXPERIMENTAL DIABETES

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This work is devoted to the morphometric assessment of structural changes in the duodenal wall of rats with skin burn injury under conditions of experimental diabetes mellitus. The control group included 21 intact animals without any signs of somatic pathology, experimental group I consisted of 21 rats with experimental skin burn injury, experimental group II consisted of 21 rats without skin burns but with experimentally simulated diabetes mellitus, and experimental group III consisted of 21 rats with both skin burn injury and experimentally simulated diabetes mellitus. The following morphometric parameters have been studied: mucosal thickness, villi height and thickness, crypt depth and width, thickness of lamina muscularis of mucosa, submucosa thickness, muscular layer thickness, serosa thickness, height of the epitheliocytes in the middle part of the villi, mitotic index of columnar epitheliocytes. The obtained data confirm the progredient course of changes characteristic of diabetic enteropathy, which gradually worsen after 7, 14 and 21 days of the experiment.

Key words: skin burn injury, streptozotocin-induced diabetes mellitus, duodenal wall, morphometric assessment.

The work is a fragment of the research project "Morphological features and changes of the digestive system organs in experimental skin burn injury", state registration No. 0119U101618.

Burn injuries and related complications are becoming more common in the current conditions of widespread use of thermal energy in production and everyday life [10, 11]. Pathogenesis of diabetes mellitus and related pathology of the digestive system are also a topical issue for present day medicine [7]. It should be noted that in the global breakdown of general injuries [12, 15] skin burn injuries accompanied by changes in the internal organs prevail [5, 1], and are the subject of current experimental studies [2, 3, 4, 6, 9]. In general, severe burns cause burn disease, with diabetic enteropathy being its component manifesting itself as intestinal dysfunction [6, 7]. However, the morphometric study of the structural features of the duodenal wall in skin burns in terms of its association with diabetes has not been the subject of special studies so far.

The purpose of the study was to perform morphometric assessment of structural changes in the duodenal wall of rats with skin burns in the conditions of experimental streptozotocin-induced diabetes mellitus.

Materials and methods. The study was performed on 84 laboratory white sexually mature male rats weighing 180-210 g. The control group was formed of 21 intact animals without signs of somatic pathology, experimental group I consisted of 21 rats with experimentally simulated skin burn injury, experimental group II - of 21 rats without skin burns with experimentally simulated diabetes mellitus, and experimental group III - of 21 rats with skin burns and experimentally simulated diabetes mellitus. The keeping of rats and all manipulations with them were carried out in full compliance with the recommendations of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986); the provisions of the European Council

Directive 86/609 / EEC (1986); requirements of the Law of Ukraine № 3447 - IV "On protection of animals from cruel treatment"; standards of "General ethical principles of animal experiments", approved by the First National Congress of Ukraine on Bioethics (Kyiv, 2001). The model of experimental diabetes mellitus [13] was simulated by a single intraperitoneal administration of streptozotocin to rats at a dose of 50 mg / kg. Streptozotocin was pre-dissolved in 0.1 M citrate buffer solution (pH-4.5). The reference value for the development of hyperglycemia in rats of experimental group II was blood glucose level of 24.24 ± 0.79 mmol / L (in the control group it was 8.03 ± 0.4 mmol / L). Burns to the skin were inflicted following the generally accepted model of F.C. Regas, H.P. Ehrlich [14], modified by I. Gunas et al. [8]. According to this model two copper plates had been pre-soaked in water at a constant temperature of 100°C for 10 minutes and then, under conditions of ether anesthesia, were tightly pressed to 2 symmetrical pre-shaved dorsal areas of the rat body for 10 seconds at a time. Burn damage to the rats' skin corresponded to II-A or II-B degree of superficial burn and covered 21-23% of the total body surface area, which causes the initiation of signs of burn shock. In a number of studies [2, 3, 4, 6, 9] it was proved that adherence to this model of experimental skin burn resulted in emergence of such characteristic signs of burn disease as endogenous intoxication, multiorgan dysfunction, generalized catabolic reaction, systemic inflammatory and systemic apoptotic response. For histological and morphometric studies a fragment of the duodenum was harvested and its biotates processed by conventional methods of light microscopy. Paraffin blocks were prepared and their sections stained with H&E. The main criteria for evaluating duodenal wall damage were the results of histological and morphometric data assessment over time, i.e. 7, 14, and 21 days after skin burns. In the above terms, rats of the respective groups were sacrificed by way of a single intraperitoneal administration of a large dose of sodium thiopental and subsequent decapitation.

For integral objective assessment of the course of compensatory, adaptive and destructive processes in the rats' duodenal wall in the setting of the conducted experiment, a morphometric study was performed using a system of visual analysis of histological slides. Images from histological slides were displayed on a computer monitor from a MICROMed SEO SCAN microscope and a Vision CCD Camera. Morphometric studies were performed using VideoTest-5.0, KAAPA Image Base and Microsoft Excel on a personal computer. The study of H&E stained slides was performed within the identified timeline of the experiment. Mucosal thickness, villi height and thickness, crypt depth and width, thickness of lamina muscularis of mucosa, submucosal thickness, muscular layer thickness, serosa thickness, height of the epitheliocytes in the middle part of the villi, mitotic index of columnar epitheliocytes were evaluated.

The values of the arithmetic mean (M), the arithmetic mean error (m) and the standard deviation (Σ) were calculated for all morphometric parameters. Significance of differences between the independent quantitative values was determined by the normal distribution (evaluation of the type of distribution was checked by Pearson's test) by Student's t-test (Bonferonni correction was used when comparing more than two groups), and in other cases by Mann-Whitney U-test. Differences at $p < 0.05$ were considered significant.

Results of the study and their discussion. Histological examination revealed the most significant changes in the rats' duodenal mucosa (RDM) in all groups. RDM villi were in the state of general polymorphism caused by their deformation and discomplexation. Processes of destructive and adaptive restructuring of the villi took place against the background of concomitant edema of loose connective tissue of the lamina propria, and leukocyte and lymphocyte infiltration (fig.1, 2).

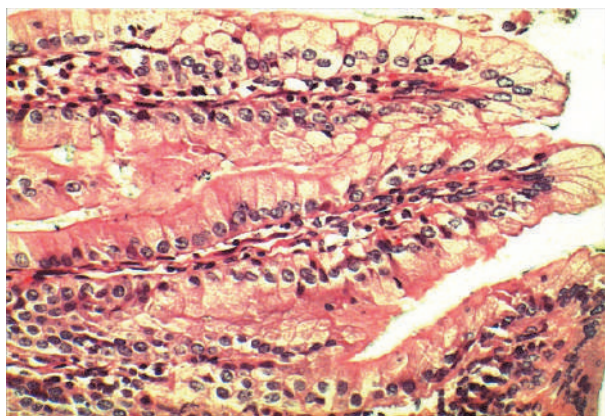


Fig. 1. Edema, leukocyte and lymphocyte infiltration of the duodenal mucosa villi of a rat from experimental group II 14 days after the start of the experiment. Microphotograph. H&E staining. Magnification 20000

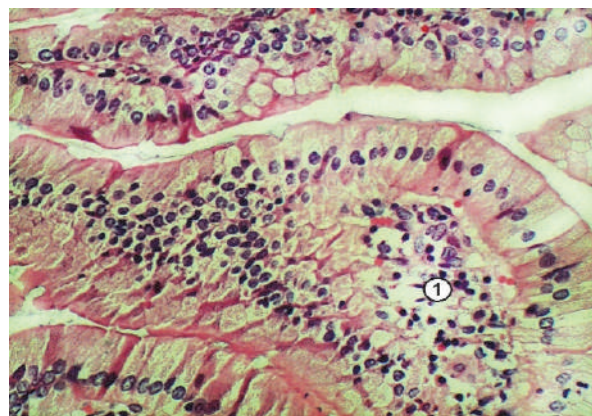


Fig. 2. Edema, leukocyte and lymphocyte infiltration of the duodenal mucosa villi of a rat from experimental group II 21 days after the start of the experiment. 1 - area of necrosis. Microphotograph. H&E staining. Magnification 200

Desquamation of columnar epitheliocytes at the apices of the RDM villi is widespread; number of goblet cells is increased; lumens of intestinal crypts are unevenly expanded; the number of typical acidophilic granules in the swollen cytoplasm of Paneth cells is reduced. Most blood vessels are dilated; there is erythrocyte stasis in the venous vessels and paravascular interstitial edema with loci of diapedetic hemorrhages and leukocyte infiltrates. Central lymphatic vessels of the villi are in the state of lymphocytosis with dilated lumen.

Table 1

Morphometric parameters of the duodenal structural components of animals of the intact group and animals with burns (experimental group I) at different terms of observation after thermal injury

Parameter (M±m)	Intact animals, n≥30	Animals with burn skin injury, n≥30		
		Day 7	Day 14	Day 21
Thickness of the mucous membrane, μm	762.51±12.35	775.63±15.49	783.76±13.46	769.77±18.89
Height of the villi, μm	581.58 ±14.23	593.06±16.86	598.09±14.87	587.51±18.03
Thickness of the villi, μm	69.30±1.97	75.27±1.98*	78.08±1.40***	77.09±2.26*
Crypt depth, μm	171.26±7.40	169.26±3.14	168.36±4.38	169.03±3.52
Crypt width, μm	38.87±1.78	40.11±0.87	40.87±1.03	39.92±1.15
Thickness of the lamina muscularis of mucosa, μm	15.05±0.20	15.12±0.17	15.17±0.26	15.11±0.28
Submucosa thickness, μm	185.45±6.08	201.80±7.80	215.64±8.36**	210.38±10.36*
Muscular layer thickness, μm	82.61±2.57	81.62±1.14	83.26±1.38	83.11±1.34
Serosa thickness, μm	7.36±0.12	7.49±0.15	7.42±0.17	7.39±0.16
Height of epitheliocytes in the middle part of the villi, μm	17.21±0.21	18.11±0.46	18.01±0.22*	17.97±0.23*
Mitotic index of columnar epitheliocytes %	3.367±0.147	2.667±0.111***	2.800±0.111**	2.867±0.171*

Notes: An asterisk indicates values that are significantly different statistically from those of the intact animals group (* – p<0.05; ** – p<0.01; *** – p<0.001).

In 14 days manifestations of cellular discomplexation, deformation and polymorphism of the RDM villi were revealed in group III; as well as areas of necrosis and apoptosis, columnar epitheliocytes which are typical for branched villi, and loss of typicality - for the shortened villi. The epithelial coating of the villi is flattened, the nuclei of epitheliocytes are shifted from the basal pole to the center of the cytoplasm. There are single lymphocytes between the epitheliocytes; shapes and sizes of goblet cells in the RDM villi are numerous and variable. The depth of intestinal crypts is reduced, their lumens are unevenly expanded and have eosinophilic content. Closer to the bottom of the crypts in the epithelial monolayer, poorly differentiated cells at different stages of mitosis were found. The cytoplasm of columnar epitheliocytes is swollen. The connective tissue of RDM lamina propria is swollen with areas of focal diapedetic hemorrhages near hemocapillaries and venules with the phenomena of stasis and sludging of erythrocytes in the vascular lumens.

Table 2

Morphometric parameters of the duodenal structural components of the intact animals group and animals with experimental streptozocin-induced diabetes mellitus (experimental group II) at different observation terms

Parameter (M±m)	Intact animals n≥30	Experimental group II, n≥30		
		Day 7	Day 14	Day 21
Thickness of the mucous membrane, μm	762.51±12.35	776.28±14.58	782.32± 5.02	784.70±16.17
Height of the villi, μm	581.58±14.23	594.66 ±13.58	605.05±19.51	607.57±11.86
Thickness of the villi, μm	69.30±1.97	78.36±1.85**	81.48±2.13***	82.80±1.86***
Crypt depth, μm	171.26±7.40	163.44±4.44	161.18±4.55	159.66±5.80
Crypt width, μm	38.87±1.78	41.94±1.46	43.83±1.52*	44.87±1.38**
Thickness of the lamina muscularis of mucosa, μm	15.05±0.20	15.10±0.42	15.30±0.24	15.36±0.27
Submucosal thickness, μm	185.45±6.08	216.47±9.63**	227.12±10.05***	237.03±10.82***
Muscular layer thickness, μm	82.61±2.57	81.59±2.97	83.65±1.48	83.87±2.98
Serosa thickness, μm	7.36±0.12	7.52±0.31	7.48±0.29	7.41±0.21
Height of epitheliocytes in the middle of the villi μm	17.21±0.21	18.29 ±0.26**	18.34±0.29**	18.39±0.19***
Mitotic index of columnar epitheliocytes %	3.367±0.147	1.967±0.102***	1.833±0.118***	1.700±0.085***

Notes: An asterisk indicates values that are significantly different statistically from those of the intact animals group (* – p<0.05, ** – p<0.01; *** – p<0.001).

In 21 days in group III RDM the phenomenon of deformation of villi was found, as a consequence of discomplexation (violation of the cellular elements ratio). Structural rearrangement with a pronounced polymorphism of villi and the appearance of adjacent villi with sharply different features were found.

Morphometric study confirmed the described qualitative changes in RDM. Quantitative signs of RDM reorganization were noted: statistically significant changes in the villi thickness, crypt width, submucosal thickness, epitheliocyte height in the middle part of villi and mitotic index of columnar epitheliocytes (Tables 1,2,3). These values are statistically significantly different from those of the intact group animals, and in animals of group II the thickness of the duodenal submucosa is $235.28 \pm 8.60 \mu\text{m}$ and is not only statistically significantly different ($p < 0.001$) from animals of the intact group ($185.45 \pm 6.08 \mu\text{m}$) and group I ($p < 0.005$). This value is the highest in animals of group III after 21 days ($237.03 \pm 10.82 \mu\text{m}$ with the significance of difference from group II $p < 0.005$). The most pronounced is the dynamic pattern of changes in the thickness of the villi and the mitotic index of columnar epitheliocytes. The mitotic index of columnar epitheliocytes of group III rats after 21 days is $1,700 \pm 0,085\%$, which is statistically significantly less ($p < 0,001$) than the identical value of rats from the intact group ($3,367 \pm 0,147\%$) and group I ($2,867 \pm 0,171\%$).

Table 3

Morphometric parameters of the duodenal structural components of animals of the intact group and animals with burn skin injury and concomitant streptozotocin-induced diabetes mellitus (experimental group III) at different observation terms

Parameter (M±m)	Intact animals n≥30	Animals with thermal skin injury + diabetes, n≥30		
		Day 7	Day 14	Day 21
Thickness of the mucous membrane, μm	762.51±12.35	772.68±17.48	780.17±12.85	782.64±14.12
Height of the villi, μm	581.58 ±14.23	592.01±15.61	600.86 ±14.08	602.54±19.05
Thickness of the villi, μm	69.30 ±1.97	78.13±1.93**	79.63±2.19***	81.08±1.79***
Crypt depth, μm	171.26 ±7.40	165.53±3.37	163.75±5.15	163.02 ±3.02
Crypt width, μm	38.87±1.78	41.76 ±1.29	42.50 ±1.49	44.04 ±1.56*
Thickness of lamina muscularis of mucosa, μm	15.05±0.20	15.11±0.25	15.25±0.40	15.27±0.26
Submucosal thickness, μm	185.45±6.08	211.88±8.41*	224.18±11.79**	235.28±8.60***
Muscular layer thickness, μm	82.61±2.57	80.77±2.20	83.67±2.14	83.72±1.35
Serosa thickness, μm	7.36±0.12	7.47±0.17	7.44±0.24	7.36±0.19
Height of epitheliocytes in the middle part of the villi μm	17.21 ± 0.21	18.22 ± 0.33*	18.35 ± 0.27**	18.37±0.23***
Mitotic index of columnar epitheliocytes %	3.367±0.147	2.400±0.123***	2.133±0.104***	2.067±0.135***

Notes: An asterisk indicates values that are significantly different statistically from those of the intact animals group (* – $p < 0,05$; ** – $p < 0,01$; *** – $p < 0,001$).

According to the scientific literature, diabetes and its complications are quite common. In the review article [11], the authors emphasize new perspectives and the need to study the pathogenetic factors of diabetic enteropathy. The data obtained by us indicate that structural transformations of RDM greatly contribute to the development of diabetic enteropathy with worsening in association with diabetes mellitus. A possible common trigger for changes is a pronounced catabolic response and ER stress in RDM cells [3].

The practical value of our data is the recommendation to include people with diabetes into a separate combustiological risk group (the possibility of enteropathy development, disruption of the intestinal epithelial barrier of the mucous membrane - translocation of the intestinal microbiota and other intraluminal content of toxic and immunogenic action).

Conclusion

The results of the conducted studies have shown the structural changes in the duodenal wall of rats with skin burn injuries to be based on deep structural transformations of its mucous membrane and to worsen in case of association of the burn injury with diabetes. Statistically significant changes of such parameters as villi thickness, crypt width, mitotic index of columnar epitheliocytes and submucosal thickness of the duodenal wall confirm the progredient course of changes characteristic for diabetic enteropathy, which gradually worsens on days 7, 14 and 21 of the experiment.

Prospects for further research in this area are related to the simulation of burn diabetic enteropathy and the study of the effects of drugs that eliminate the catabolic reaction caused by intoxication on the condition of experimental laboratory animals.

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Реферати

МОРФОМЕТРИЧНИЙ АНАЛІЗ СТРУКТУРНИХ ЗМІН СТІНКИ ДВНАДЦЯТИПАЛОЇ КИШКИ ЩУРІВ ПРИ ОПІКОВІЙ ТРАВМІ ШКІРИ ЗА УМОВ ЕКСПЕРИМЕНТАЛЬНОГО ЦУКРОВОГО ДІАБЕТУ

Тимошенко І.О., Черкасов Е.В., Шепітько К.В.

Робота присвячена морфометричному аналізу структурних змін стінки дванадцятипалої кишки щурів при опіковій травмі шкіри за умов експериментального цукрового діабету. Групою контролю була 21 інтактна тварина без ознак соматичної патології, I експериментальну групу склали 21 щур з експериментальною опікою травмою шкіри, II експериментальну групу склали – 21 щур без опіку шкіри з експериментально відтвореним цукровим діабетом, III експериментальну групу склали – 21 щур з опіковою травмою шкіри та експериментально відтвореним цукровим діабетом. Експериментальний цукровий діабет моделювали шляхом внутрішньочеревного введення щурам стрептозоточина одноразово в дозі 50 мг / кг. Опікове ушкодження шкіри у щурів відповідало II - А чи II - Б ступеня - поверхневого опіку площею 21-23% загальної поверхні тіла з розвитком опікового шоку. Були досліджено наступні морфометричні показники: товщину слизової оболонки, висоту ворсинок, товщину ворсинок, глибину крипт, ширину крипт, товщину м'язової пластинки слизової оболонки, товщину підслизової основи, товщину м'язової оболонки, товщину серозної оболонки, висоту епітеліоцитів в середній частині ворсинок, мітотичний індекс стовпчастих епітеліоцитів. Одержані дані підтверджують прогресивний перебіг характерних для діабетичної ентеропатії змін, поглиблення яких поступово збільшується через 7, 14 та 21 добу експерименту.

Ключові слова: опікова травма шкіри, стрептозоточиніндукований цукровий діабет, стінка дванадцятипалої кишки, морфометричний аналіз.

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МОРФОМЕТРИЧЕСКИЙ АНАЛИЗ СТРУКТУРНЫХ ИЗМЕНЕНИЯХ СТЕНКИ ДВЕНАДЦАТИПЕРСТНОЙ КИШКИ КРЫС ПРИ ОЖОГОВОЙ ТРАВМЕ КОЖИ В УСЛОВИЯХ ЭКСПЕРИМЕНТАЛЬНОГО САХАРНОГО ДИАБЕТА

Тимошенко И.А., Черкасов Е.В., Шепитько К.В.

Робота посвящена морфометрическому анализу структурных изменений стенки двенадцатиперстной кишки крыс при ожоговой травме кожи в условиях экспериментального сахарного диабета. Группой контроля была 21 интактная животная без признаков соматической патологии, I – экспериментальную группу составили 21 крыса с экспериментальной ожоговой травмой кожи, II – экспериментальную группу составили – 21 крыса без ожога кожи с экспериментально воспроизведенным сахарным диабетом, III экспериментальную группу составили – 21 крыса с ожоговой травмой кожи и экспериментально воспроизведенным сахарным диабетом. Экспериментальный сахарный диабет моделировали путем внутрибрюшинно введением крысам стрептозоточина однократно в дозе 50 мг / кг. Ожоговое повреждение кожи у крыс соответствовало II – А или II – Б степени - поверхностного ожога площадью 21-23% общей поверхности тела с развитием ожогового шока. Были исследованы следующие морфометрические показатели: толщину слизистой оболочки, высоту ворсинок, толщину ворсинок, глубину крипт, ширину крипт, толщину мышечной пластинки слизистой оболочки, толщину подслизистой основы, толщину мышечной оболочки, толщину серозной оболочки, высоту эпителиоцитов в средней части ворсинок, митотический индекс столбчатых эпителиоцитов. Полученные данные подтверждают прогрессивное течение характерных для диабетической энтеропатии изменений, углубление которых постепенно увеличивается через 7, 14 и 21 сутки эксперимента.

Ключевые слова: ожоговая травма кожи, стрептозоточининдуцированный сахарный диабет, стенка двенадцатиперстной кишки, морфометрический анализ.

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MICROENVIRONMENT OF INTESTINAL ANASTOMOSES WITH COMPARATIVE ASSESSMENT OF IMMUNE STATUS IN PATIENTS WITH SMALL INTESTINE ANASTOMOSES

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The purpose of the study was to assess local immune status as a marker for the risk of intestinal anastomosis failure. The research was based on the study of local immune mechanisms of intestinal anastomoses failure in the comparative aspect. The material of the study was the anastomosis tissue, areas of the intestine at a distance of 1-3 cm from the anastomosis, as well as the patients' blood samples taken before surgery, on the first and the fifth days after the formation of the anastomosis in subgroups of the study. The occurrence of intestinal anastomoses failure is mainly determined by the interaction of immunocompetent cells and products of their activity - cytokines. Immunological disorders in the intestine tissues in the anastomosis area cause the failure pathogenesis. The microenvironment of intestinal anastomoses by immunological parameters is a field that promotes further progression of inflammation or serves as a buffer zone that protects tissue from pathological changes. The study revealed the formation of secondary immune deficiency in patients with resection of intestinal segments; recovery of immune status indices in patients with uncomplicated postoperative period occurs at an earlier date and to the fuller extent.

Key words: intestinal anastomoses, failure, immunology.

The work is a fragment of the initiative research project "To develop technologies for the prediction, prevention and surgical treatment of anastomotic failure in patients with small bowel surgery" at the stage of planning.

An intestinal anastomosis is a surgical procedure performed to establish a connection between two previously distant parts of the intestine. This procedure restores the continuity of the intestine after the removal of a pathological condition that affects the intestine [3].

The history of the intestinal suture study goes back over 200 years and it is no secret that great progress has been made in this field of abdominal surgery. Numerous clinical and experimental studies provide various information on the efficacy of an intestinal suture's particular type. It is known that the main pathophysiological aspect in patients with intestinal anastomoses (IA) failure are volemic and hemodynamic disorders, which are caused by reduced arterial inflow and impaired venous outflow due to compression of intramural vessels, sequestration of fluid into the lumen of the intestine and abdominal cavity [3, 4].

Endogenous intoxication and metabolic disorders in IA failure are of complex and multicomponent nature [2, 6]. The main source of endotoxemia in patients with IA failure is the intestine [5]. Impairment of the intestinal wall's barrier function leads to endotoxemia, which is progressive in the absence of adequate treatment [4, 5, 9].

Chronic inflammation to some extent may serve as a background for the intestinal anastomoses failure against the background of proliferation in the intestinal mucosa. The starting point for the proliferation are the epithelial cells of the intestinal mucosa [1]. The immune system of the gastrointestinal tract (GIT) is represented by lymphoid tissue associated with the intestine (GALT-gut associated lymphoid tissue), located in three parts - diffusely distributed by its own plate under the intestinal epithelium, in the epithelium and in organized lymph follicles, such as Peyer's patches. It is in close contact with a huge flow of microbial and allergenic material coming from the intestinal lumen, and serves as the first barrier in its path [9].

The following factors are crucial in the further development of pathological changes: gradual depletion of the liver detoxification potential; translocation of intestinal microflora and its acquisition of pathogenic properties; growth in the total mass of toxic products in internal environments; development of systemic microcirculatory disorders in organs and tissues, disorders of cellular metabolism; development and progression of peritonitis, the second source of intoxication [6, 9].

In our opinion (and this is confirmed by various studies [1, 7, 8, 9]), it is important to study the role of immunocompetent cells and cytokines in the formation of the intestinal anastomoses failure.

The purpose of the study was to assess the local immune status as a marker for the risk of intestinal anastomoses t failure.

Materials and methods. The work was based on the study of local immune mechanisms of intestinal anastomoses failure in the comparative aspect. The material of the study was the anastomosis tissue, as well as areas of the intestine at a distance of 1-3 cm from the anastomosis. Clinical material (paraffin blocks)

sampled from 52 patients who were selected by random sampling, who were performed resections of small bowel segments with the formation of intestinal anastomoses, as well as blood samples taken before surgery, on the first and the fifth days after the formation of the anastomosis, in SI "V.T. Zaitsev Institute of General and Urgent Surgery of NAMSU" in the period from 2001 to 2018. The mean age of patients was 54.7 ± 5.9 years. Of all the operated there were - 29 (55.9%) women and 23 (44.1%) men.

The patients were divided into two subgroups: subgroup I consisted of patients with uncomplicated postoperative period (23 patients), subgroup II included 29 patients with complicated postoperative period (who were diagnosed with intestinal anastomoses failure).

Immunological typing of tissue lymphocytes (CD3 +, CD3 + CD4 +, CD10 + CD8 +, CD16 + CD56 +, CD19 +, T-lymphocytes with receptors) and cytokines (TNF-, IL-1, IL-2, IL-6, IL-8, IL-10), as well as studies of markers (antibodies): Vimentin, Ki - 67, were evaluated using monoclonal antibodies by immunohistological staining with Ventana Medical Systems, Inc. automatic system using Ventana kits (Ventana, Tucson, AZ) in compliance with the manufacturer's instructions.

Serial paraffin sections were prepared and applied to adhesive-coat glass. The study was performed according to the method of C.R. Taylor, R. Cote. Pre-treatment of the sections was performed by the method of restoration of antigenic tissue determinants (S.H Shi, 1991 and M. Sowa; Y. Kato, I. Nakanishi et al., 1992). Incubation with primary antibodies lasted 30 min at room temperature. The sections were thoroughly washed in the buffer, dried, and then treated with the En Vision system (Daco, Denmark). Monoclonal antibodies were to desmin (Daco, RTU dilution), to vimentin (Daco, RTU dilution), to Ki - 67 (Daco, RTU dilution). The study was performed at different types and methods of anastomoses formation.

The study of the total population of T-lymphocytes (CD3⁺), subpopulations of T-lymphocytes - T-helpers (CD4⁺), T-suppressors (CD8⁺) and β -lymphocytes (CD19⁺ and CD20⁺) in blood serum was performed using monoclonal antibodies CD3⁺, CD4⁺, CD8⁺, CD19⁺ and CD20⁺ by the immunofluorescence method with STAT-FAX303 enzyme-immunoassay analyzer, USA. The content of immunoglobulins A, M, G (IgA, IgM, IgG), total immunoglobulin E (IgE) in the blood serum was studied using enzyme-immunoassay systems produced by Tov. NVL "Granum-Ukraine", and the content of allergen-specific IgE was studied using enzyme-immunoassay systems produced by NVT "Microgen" (Russia). Studies of circulating immune complexes were performed in blood serum by the method of Gashkova et al. (1977), as well as tumor necrosis factor alpha (TNF- α) in the blood serum was detected using enzyme-immunoassay systems manufactured by VAT "Protein Contour" (Russia) and "Diacclone" (France).

Statistical analysis was performed using the modern software package STATISTICA 7.0 (StatSoft Inc., USA) and MedCalc (version 9.3.5.0).

Results of the study and their discussion. Assessment of local immune status in the intestinal anastomoses failure revealed that the content of T-helper-induction lymphocytes (CD3⁺, CD4⁺) in the tissue of the anastomosis was reliably ($p \leq 0.05$) higher by 44.7%. Respectively, the CD4⁺ / CD8⁺ index was by 1.7 times and by 55.0% higher than that of relatively healthy tissue ($p < 0.05$), but the number of CD8⁺ cells and B lymphocytes was reduced by 21 % and 23.5 % respectively compared to relatively healthy tissue and by 42.2% (table 1).

In the intestinal tissue, which was sampled at the distance of 1-3 cm from the anastomosis, on the contrary, there was a higher content of CD19⁺ cells by 37.2%, but also a lower number of cytotoxic cells (CD8⁺) (18.0%) compared to the indices along the line of resection ($p < 0.05$) (table. 1). According to other criteria, no reliable differences were found. Studies of local immune status in different parts of the intestine did not reveal significant differences.

Table 1

Immunohistochemical parameters of lymphocyte subpopulation in intestinal tissues of patients with intestinal anastomoses in subgroups

Subpopulation of lymphocytes	Patients of group I			Patients of group II		
	line of resection	1-3 cm from anastomosis	7-10 cm from anastomosis	line of resection	1-3 cm from anastomosis	7-10 cm from anastomosis
CD3 ⁺	76.2 \pm 3.1	57.5 \pm 2.8*	71.6 \pm 2.6*	65.3 \pm 1.6*	58.7 \pm 3.0	51.4 \pm 2.2*•
CD3 ⁺ / CD4 ⁺	40.7 \pm 2.4*•	24.5 \pm 3.1*•	31.1 \pm 2.7*	34.8 \pm 1.5*	24.2 \pm 1.4*	23.3 \pm 1.2*•
CD3 ⁺ / CD8 ⁺	34.8 \pm 2.0	31.3 \pm 2.6	35.3 \pm 2.1*	29.0 \pm 1.3 *	28.5 \pm 1.7*	35.6 \pm 2.5*•
CD16 ⁺ / CD56 ⁺	9.2 \pm 2.1	20.5 \pm 2.2*	12.4 \pm 1.6	12.1 \pm 1.6	11.1 \pm 1.5	10.1 \pm 1.2*
CD19 ⁺	12.2 \pm 2.4	20.1 \pm 1.5*•	16.3 \pm 2.4*	17.3 \pm 2.1 *	31.8 \pm 2.0 *	24.0 \pm 1.8*
CD4 ⁺ / CD8 ⁺	1.9 \pm 0.33	0.95 \pm 0.21*	1.15 \pm 0.36	1.6 \pm 0.25*	1.14 \pm 0.26	0.97 \pm 0.13*•
CD10 ⁺	21.2 \pm 1.8	16.5 \pm 1.45*	24.1 \pm 2.0	11.5 \pm 1.3	9.5 \pm 0.88*	12.4 \pm 1.1

Note - statistically significant compared to the resection line (7-10 cm - conditionally healthy tissue): * - by Student's test, $p < 0.05$; • - by the Z-criterion, $p < 0.05$

A study was performed on the CD10 immunohistochemistry relevance for diagnosis in this clinical situation, isolating the intestinal mucosa. Uniformly positive CD10-immunostaining was detected in the normal intestinal mucosa, but variable expression loss was revealed under the conditions of intestinal anastomosis failure. In particular, CD10 staining was lost in 80% of the mucosa in intestinal anastomoses failure, usually in the presence of active inflammation. There was no expression of CD10 in normal intestinal mucosa 7-10 cm from the anastomosis. Therefore, although CD10 immunostaining identifies normal intestinal mucosa with 100% specificity, negative staining does not definitively exclude anastomotic failure.

In general, in patients with intestinal anastomoses failure, the process was characterized by a high content of tumor-infiltrating T-helper-inducer lymphocytes ($CD3^+$, $CD4^+$, $CD3^+ / CD4^+$, $CD3^+ / CD8^+$) and low levels of cytotoxic ($CD3^+$, $CD8^+$ and $CD19^+$). However, the resection line may have maintained an immune barrier due to the increased content of B-lymphocytes ($CD19^+$) compared to the intact tissue.

Cytokines are known to be the major products of immunocompetent cells. We have studied the level of tissue cytokines (IL-2, TNF- α , IL-6, IL-8, IL-10, IL-1) in the intestinal anastomosis and in the surrounding tissues (table 2).

Table 2

Immunohistochemical parameters of interleukins in intestinal tissues of patients with intestinal anastomoses in subgroups

Subpopulation of interleukines	Subgroup I (n=23)			Subgroup II (n=29)		
	line of resection	1-3 cm from anastomosis	7-10 cm from anastomosis	line of resection	1-3 cm from anastomosis	7-10 cm from anastomosis
IL-1	43.3 \pm 4.1*•	49.9 \pm 5.16*	58.5 \pm 6.4*•	270.3 \pm 25.5*•	80.29 \pm 9.95*•	100.12 \pm 13.1*
IL-2	35.7 \pm 5.1*	32.6 \pm 3.7*•	60.3 \pm 6.6*•	18.1 \pm 3.31*	7.65 \pm 1.42*	5.55 \pm 0.74*•
IL-6	21.9 \pm 4.7*	25.5 \pm 5.1	39.11 \pm 8.4*	190.9 \pm 23.1*•	16.8 \pm 1.22*•	55.21 \pm 4.6*•
IL-8	181.1 \pm 16.7*	166.2 \pm 17.7*	169.4 \pm 13.3*	520.1 \pm 19.0*•	242.5 \pm 20.4*	130.2 \pm 15.7*•
IL-10	26.6 \pm 2.5*	15.5 \pm 1.8*	17.4 \pm 1.9*	8.56 \pm 1.94	6.22 \pm 1.55*	8.24 \pm 1.75
TNF- α	7.08 \pm 1.13	5.9 \pm 1.05	5.33 \pm 0.91*	17.32 \pm 1.12	11.73 \pm 1.51*	12.07 \pm 0.65*

Note. Statistically significant for the resection line (7-10 cm - conditionally healthy tissue): * - by Student's test, $p < 0.05$; • - by the Z-criterion, $p < 0.05$

Generally, all the studied cytokines in patients with intestinal anastomoses failure had a high level, except for IL-10. Thus, the content of IL-2, IL-6, IL-8, IL-1 in tumor tissue was by 3 times higher than compared to the resection area. In the area of the resection line, the level of IL-8 was statistically reliably ($p < 0.05$) by 2 times lower compared to the area of 1-3 cm from the anastomosis (table 2).

Analysis of the distant intestinal mucosa obtained from patients with intestinal anastomoses failure, revealed the presence of the crypts in the basal parts, proliferation of Ki67 positive cells, and single cells that expressed Vim and were located among the epithelium of the crypts (fig. 1).

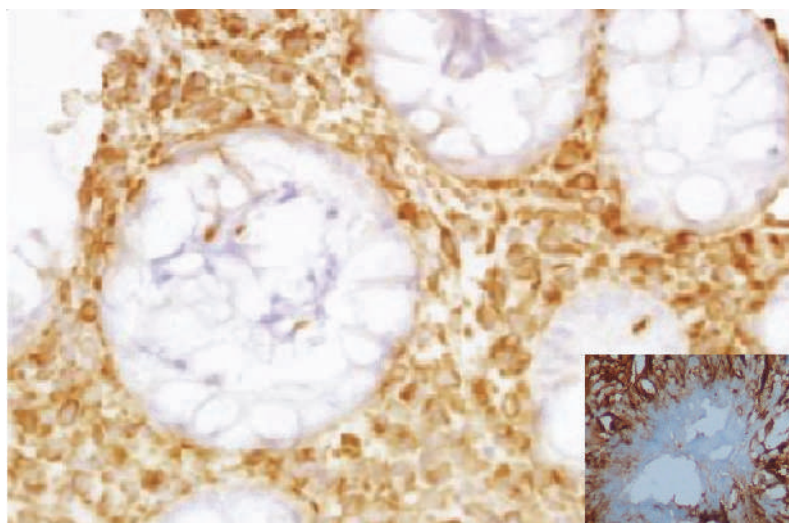


Fig. 1. Immunohistochemical expression of Vimentin in the intestinal mucosa of patients with intestinal anastomoses failure.

As a result the immunohistochemical examination of the mucous membrane revealed differences in the main and the control groups. Thus, the proliferation index for Ki67 in group II with intestinal anastomoses failure was 88.42%, in group I (control) - 0% ($p = 0.0001$) Vimentin.

When studying the initial parameters of immune status in patients of the studied subgroups, before surgery and on the first day after surgery ($p > 0.05$), reliably significant differences were revealed. It was found that in patients with complicated postoperative period (subgroup II) immunogram values were reliably ($p \leq 0.05$) lower. In the uncomplicated course of the postoperative period (subgroup I), this index is almost close to normal values ($63.47 \pm 0.68\%$), while in subgroup II by the end of treatment there was only a tendency to increase the content of $CD3^+$ in the blood ($55.53 \pm 0.65\%$).

When determining the level of CD20⁺ in the patients of both subgroups it was revealed almost twofold increase in this index compared to normal values, but in patients with uncomplicated postoperative period there was a more pronounced tendency to normalize the level of CD20⁺ in patients of subgroup I (22.6±0.29%) than in patients of subgroup II (25.67±0.37%, p < 0.05).

In addition, there was a reliable (p < 0.05) increase in the level of NK cells, which may be associated with an impairment of the intestinal mucosa barrier function and with the penetration of the intestinal flora antigens into the submucosal layer. The results of the lymphocyte subpopulations study are presented in table 3.

Table 3

Dynamics of lymphocyte subpopulations and phagocytic activity of neutrophils in peripheral blood samples of patients in subgroups under study

Indices	Norm, %	Subgroup I (n=23)		Subgroup II (n=29)	
		Day 1,%	Day 5,%	Day 1,%	Day 5,%
CD3 ⁺ (T-lymphocytes)	66.2±0.5	47.12±0.91*	63.47±0.68	50.93±0.83*	55.53±0.65**
CD4 ⁺ (T-helpers)	43.9±0.8	29.12±0.61*	40.51±0.46	30.77±0.6*	33.3±0.66**
CD8 ⁺ (T-cytotoxic)	27.0±0.9	15.21±0.35*	22.11±0.5	16.2±0.3*	18.45±0.41
CD16 ⁺ (NK-cells)	13.5±0.7	19.12±0.45*	12.56±0.34	18.97±0.41*	14.73±0.32
CD20 ⁺ (B- lymphocytes)	14.0±0.2	27.4±0.27*	22.6±0.29	27.67±0.25*	25.67±0.37**
CD4 ⁺ /CD8 ⁺	1.9±0.02	1.92±0.18	1.86±0.15	1.87±0.16	1.7±0.15
Neutrophil phagocytic activity (%)	66.32±2.11	46.68±1.14*	64.2±1.65	45.23±0.89*	53.03±1.03**
Phagocytic number	5.5±0.4	2.77±0.05*	4.72±0.11	2.80±0.06*	3.85±0.1**

Note: * p < 0.05 compared to normal values, ** p < 0.05 compared to subgroup I.

In the examined patients a significant decrease in neutrophil phagocytic activity and in phagocytic number was revealed. Faster and more complete recovery of phagocytic activity (64.2±1.65%) and phagocytic number (4.72±0.11%) indices occurred in subgroup I compared to subgroup II (53.03±1.03% and 3.85±0.1%, respectively), which created the preconditions for the restoration of phagocytosis and its completion, and, consequently, to reduce the risk of complications in the postoperative period.

In patients of subgroup I there was an increase in the level of Ig A by 2 times (5.11±0.07 g / l) at a normal value of 2.5±0.08 g / l, this is due to the fact that immunoglobulins of the IgA class are "the first line of the body's defense" on the mucous membranes of the gastrointestinal tract. In the study of the level of the IgM and IgG classes immunoglobulins, no reliably (p < 0.05) significant deviations from normal values were established (table 4).

Table 4

The level of Ig A, Ig M, Ig G immunoglobulins in peripheral blood samples of patients in subgroups under study

Indices	Norm, g/l	Subgroup I (n=23)		Subgroup II (n=29)	
		Before treatment, g/l	Day 5, g/l	Before treatment, g/l	Day 5, g/l
Ig A	2.5±0.08	5.11±0.07*	3.75±0.06	4.85±0.07*	4.27±0.05**
Ig M	1.51±0.05	1.18±0.05	1.30±0.04	1.22±0.05	1.25±0.04
Ig G	14.7±0.42	16.3±0.2	14.84±0.10	15.55±0.07	15.17±0.09

Note: * p < 0.05 compared to normal values, ** p < 0.05 compared to subgroup I.

It should be noted that the state of cellular and humoral immunity in patients with small intestine surgery suffers markedly, and is characterized, to a greater extent, as general immune depression in patients with anastomotic failure, which largely correlates with studies on complicated surgical pathology [7]. Data on the T-lymphocytes reduction against the background of increased duration of the pathological process and the severity of inflammation, which we observed in patients of subgroup II, significantly correlates with literature data, and necessitates further study of the impact of immunocompetent cells on the processes of small intestinal anastomoses healing [1]. Patients examined in the study showed a decrease in phagocytic activity of neutrophils, as well as in phagocytic number, in addition, the recovery of phagocytic activity and phagocytic number was slower in subgroup II, which may be associated with activation of the immune response against infectious agents, which may be factors in gastrointestinal pathologies [9].

Conclusion

Development of intestinal anastomoses failure is largely determined by the interaction of immunocompetent cells and products of their activity - cytokines. Local immunological disorders in

intestinal tissues along the line of resection cause pathogenetic initiation of anastomosis failure. The microenvironment of intestinal anastomoses according to its immunological parameters is a field that promotes further progression of inflammation or serves as a buffer zone that protects tissue from pathological changes.

Studies have shown that in patients who underwent resection of intestinal segments, secondary immune deficiency is formed, which is determined by abnormalities in the system of cellular and humoral immunity. Earlier and complete recovery of immune status occurs in patients with uncomplicated postoperative period.

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Реферати

МІКРООТОЧЕННЯ КИШКОВИХ АНАСТОМОЗІВ З ПОРІВНЯЛЬНОЮ ОЦІНКОЮ ІМУННОГО СТАТУСУ У ПАЦІЄНТІВ З АНАСТОМОЗАМИ ТОНКОЇ КИШКИ

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Метою дослідження є оцінка локального імунного статусу як маркера ризику неспроможності кишкового анастомозу. Дослідження базувалося на основі вивчення в порівняльному аспекті локальних імунних механізмів неспроможності кишкових анастомозів. Матеріалом дослідження служили тканина анастомозу, ділянки кишки на відстані 1-3 см від анастомозу а також зразки крові пацієнтів, забрані в операції, на першу і п'яту добу після формування анастомозу в підгрупах дослідження. Виникнення неспроможності кишкових анастомозів в основному визначається взаємодією імунокомпетентних клітин і продуктів їх активності - цитокинів. Імунологічні порушення в тканинах кишки в зоні анастомозу обумовлюють патогенез неспроможності. Мікрооточення кишкових анастомозів за імунологічними параметрами є полем, сприяє подальшому прогресуванню запалення або служить буферною зоною, захищає тканину від патологічних змін. Дослідження виявило формування вторинної імунної недостатності у пацієнтів з резекцією сегментів кишечника; відновлення показників імунного статусу у пацієнтів з неускладненим післяопераційним періодом відбувається в більш ранні терміни і в більш повному обсязі.

Ключові слова: Кишкові анастомози, неспроможність, імунологія.

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МІКРООКРУЖЕННЯ КИШЕЧНИХ АНАСТОМОЗІВ СО СРАВНИТЕЛЬНОЙ ОЦЕНКОЙ ИМУННОГО СТАТУСА У ПАЦИЕНТОВ С АНАСТОМОЗАМИ ТОНКОЙ КИШКИ

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Целью исследования является оценка локального иммунного статуса как маркера риска несостоятельности кишечного анастомоза. Исследование базировалось на основе изучения в сравнительном аспекте локальных иммунных механизмов несостоятельности кишечных анастомозов. Материалом исследования служили ткань анастомоза, участка кишки на расстоянии 1-3 см от анастомоза а также образцы крови пациентов, забранные в операции, на первые и пятые сутки после формирования анастомоза в подгруппах исследования. Возникновение несостоятельности кишечных анастомозов в основном определяется взаимодействием иммунокомпетентных клеток и продуктов их активности - цитокинов. Иммунологические нарушения в тканях кишки в зоне анастомоза обуславливают патогенез несостоятельности. Микроокружение кишечных анастомозов по иммунологическим параметрам является полем, способствует дальнейшему прогрессированию воспаления или служит буферной зоной, защищает ткань от патологических изменений. Исследование выявило формирования вторичной иммунной недостаточности у пациентов с резекцией сегментов кишечника; восстановление показателей иммунного статуса у пациентов с неосложненным послеоперационным периодом происходит в более ранние сроки и в более полном объеме.

Ключевые слова: Кишечные анастомозы, несостоятельность, иммунология.

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INDICES OF THE CELL CYCLE IN THE THYROID GLAND AFTER THERMAL BURNS OF THE SKIN WHEN USING SOLUTIONS OF LACTOPROTEIN WITH SORBITOL OR HAES-LX 5 %

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The DNA content in the nuclei of thyroid cells of 90 white male rats on the background of skin burns of 2-3 degrees (with a lesion area of 21-23 % of the body surface) and the introduction of solutions of lactoprotein with sorbitol or HAES-LX 5 % was determined by flow cytometry. At 1, 3, 7 and 14 days after thermal trauma to the skin and the use of lactoprotein with sorbitol or HAES-LX 5 %, only lower values of S-phase values were found compared to the groups without burns. 21 days after thermal damage to the skin in the group with infusion HAES-LX 5 %, the interval SUB-G0G1 is significantly higher than in the control group. After 30 days in the groups with prior administration of HAES-LX 5 % and lactoprotein with sorbitol solutions, the value of SUB-G0G1 is significantly higher than that in the groups without skin burns.

Key words: thyroid gland, thermal skin burn, DNA cytometry, HAES-LX 5 %, lactoprotein with sorbitol.

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Thermal damage to the skin and its systemic manifestation – burn disease (BD) remains the focus of modern medical research on both the study of the pathogenesis of this pathological condition and the development of new therapies [8]. It is noted that the insufficient effectiveness of existing methods of therapy is due to a complex cascade of factors of this pathology, leading to systemic damage to the body with thermal skin burns [11].

Although today the main stages of BD therapy for thermal damage to the skin, which consist in intensive replenishment of lost fluid, early necrectomy, effective antibiotic therapy, have a high mortality on the background of BD, even in the long term of this pathological condition [7]. That is why there is an active search for new methods of treatment of skin burns, mostly local, with the development of new synthetic materials that promote local synthesis of keratinocytes, but their use does not prevent the reduction of systemic manifestations of BD [5].

It is indicated [14] that the complex nature of burns necessitates the need for systemic treatment of thermal damage to the skin, which should affect all pathogenetic factors of this condition. It is well known [9] that the use of systemic, pathogenetically based therapy in the earliest terms improves treatment results, increases patient survival and reduces mortality even in the long term of BD. Therefore, the treatment of burns remains a topical issue in modern medicine and combustiology, which requires the development of new therapies that will affect the level of pathogenetic factors of the disease, as existing therapeutic agents do not provide sufficient effectiveness [10].

Our attention was drawn to the data on the positive results of the use of early active infusion therapy on the background of thermal skin burns of domestic drugs – solutions of lactoprotein with sorbitol (LPS) and HAES-LX 5 %, which showed a significant positive effect on various aspects of BD, including thymus, lungs, liver and other organs [3]. The application of the method of DNA cytometry allowed to establish the patterns of pathogenetic effects of burns on the body and on the cells in the studied organs. We did not find literature data on the study of thyroid cell division by DNA cytometry on the background of BD with the introduction of infusion solutions.

The purpose of the work was to study the dynamics of cell cycle parameters and DNA fragmentation in rat thyroid cells, against the background of skin burns and the introduction of lactoprotein with sorbitol or HAES-LX 5 %.

Material and methods. Experimental studies were performed on 90 white male rats weighing 160-180 g (obtained from the vivarium of the Institute of Pharmacology and Toxicology of the National Academy of Medical Sciences of Ukraine), conducted on the basis of research laboratory of functional morphology and genetics of development of National Pirogov Memorial Medical University, Vinnytsya. The keeping and manipulation of animals was carried out in accordance with the "General Ethical Principles of Animal Experiments" adopted by the First National Congress on Bioethics (Kyiv, 2001) and was guided by the recommendations of the "European Convention for the Protection of Vertebrate Animals for Experimental and Other Scientific Purposes" (Strasbourg, 1985), methodological recommendations of

the State Pharmacological Center of the Ministry of Health of Ukraine on "Preclinical studies of drugs" (2001), as well as rules of humane treatment of experimental animals and conditions approved by the Bioethics Committee of National Pirogov Memorial Medical University, Vinnytsya (Minutes № 1 of 14.01.2010).

Infusion of LPS or HAES-LX 5 % solutions was performed into the inferior vena cava after catheterization under aseptic conditions through the femoral vein. The catheter was sutured under the skin, its lumen along the entire length was filled with titrated heparin solution (0.1 ml of heparin per 10 ml of 0.9 % NaCl solution) after each administration of substances. Infusions were performed once a day for the first 7 days. Thermal burns of 2-3 degrees skin were performed by applying four copper plates (each with a surface area of 13.86 cm²) to the pre-depilated side surfaces of the rat body for 10 seconds, which were preheated for 6 minutes in water with a temperature of 100°C [7]. The total area of skin lesions was 21-23 %. Catheterization of the main vessels, thermal skin burns and decapitation of animals (after 1, 3, 7, 14, 21 and 30 days) were performed under propofol anesthesia (60 mg/kg i/v).

The DNA content in the nuclei of rat thyroid cells was determined by flow cytometry. Under conditions of propofol anesthesia (60 mg/kg i/v) in animals after removal of all its contents, prepared nuclear suspensions for flow cytometry using a solution for nuclear DNA CyStain DNA Step 1 company Partec (Germany), according to the manufacturer's protocol-instructions. This solution allows you to quickly perform the extraction of nuclei and label nuclear DNA with diamidinophenylindole, which is part of it. Disposable CellTrics 50 µm filters (Partec, Germany) were used in the process of manufacturing nuclear suspensions. Flow analysis was performed on a multifunctional flow cytometer "Partec PAS" company Partec, Germany, in the research center of National Pirogov Memorial Medical University, Vinnytsya.

UV radiation was used to excite diamidinophenylindole fluorescence. From each sample of the nuclear suspension 10,000 events were analyzed. Cell cycle analysis was performed using FloMax software (Partec, Germany) in full digital correspondence according to a mathematical model, which determined: G0G1 – the percentage of G0G1 phase cells to all cells of the cell cycle (DNA content = 2c); S is the percentage of the phase of DNA synthesis to all cells of the cell cycle (DNA content > 2c and < 4c); G2+M is the percentage of the G2+M phase to all cells of the cell cycle (DNA = 4c). Determination of DNA fragmentation (apoptosis) was performed by isolating the SUB-G0G1 region on RN2 DNA histograms before the G0G1 peak, which indicates cell nuclei with a DNA content < 2c.

Statistical processing of the obtained results was performed in the license package "STATISTICA 6.1" using non-parametric methods of evaluation of the obtained results. The mean values of each trait, standard deviation, and percentile range were evaluated. The significance of the difference in values between the independent quantitative values was determined using the Mann-Whitney U-test for independent samples.

Results of the study and their discussion. Indices of cell cycle and DNA fragmentation of thyroid cells in rats on the background of infusion for 7 days 0.9 % solution of NaCl, LPS or HAES-LX 5 %, as well as the use of infusion of 0.9 % NaCl solution on the background of thermal skin burns are reflected in our articles [12, 13].

A similar picture of cell cycle parameters in thyroid cells 1 day after thermal damage to the skin in rats infused with 0.9 % NaCl solution, LPS or HAES-LX 5 % (table 1). Thus, against the background of the use of LPS or HAES-LX 5 % also significantly lower values of the S-phase (p<0.01), and the difference between the indices G0G1, G2+M and SUB-G0G1 was not detected.

Table 1

Indices of the cell cycle in the cells of the thyroid gland of rats 1 day after skin burns when using infusion therapy according to flow DNA cytometry data (M±σ)

Group	Indices of the cell cycle (%)			
	S	SUB-G0G1	G0G1	G2+M
0.9 % NaCl	0.652±0.134	2.462±0.800	91.16±2.41	8.192±2.368
Burn + 0.9 % NaCl	0.234±0.094	2.732±1.141	91.90±2.65	7.868±2.678
LPS	0.548±0.118	2.814±0.707	90.87±1.69	8.576±1.759
Burn + LPS	0.272±0.061	2.868±0.901	90.64±1.72	9.090±1.731
p(LPS – burn+LPS)	<0.01	>0.05	>0.05	>0.05
HAES-LX 5 %	0.638±0.162	2.688±0.870	90.68±1.93	8.682±1.855
Burn + HAES-LX 5 %	0.326±0.063	2.626±0.870	90.93±1.45	8.748±1.431
p(HAES-LX5% – burn+HAES-LX 5%)	<0.01	>0.05	>0.05	>0.05
p(burn+0.9% NaCl – burn+ LPS)	>0.05	>0.05	>0.05	>0.05
p(burn+0.9% NaCl – burn+HAES-LX 5%)	>0.05	>0.05	>0.05	>0.05
p(burn + LPS – burn+HAES-LX 5%)	>0.05	>0.05	>0.05	>0.05

3 days after skin burn on the background of the use of HAES-LX 5 % or LPS solutions, only the maximum decrease in the percentage of thyroid cells that were in the S-phase ($p < 0.01$) compared to the corresponding groups without burns (table 2) was established. Between the data of groups 0.9 % NaCl solution and LPS or HAES-LX 5 % set significantly lower ($p < 0.01$ in both cases) the value of SUB-G0G1, as well as a trend ($p = 0.060$) for larger values of S-phase when using HAES-LX 5 % compared to 0.9 % NaCl solution (table 2).

Table 2

Indices of the cell cycle in the cells of the thyroid gland of rats 3 days after skin burns when using infusion therapy according to flow DNA cytometry ($M \pm \sigma$).

Group	Indices of the cell cycle (%)			
	S	SUB-G0G1	G0G1	G2 + M
0.9 % NaCl	0.622±0.110	2.594±0.628	90.99±2.48	8.392±2.375
Burn + 0,9 % NaCl	0.214±0.105	5.288±0.840	91.46±2.80	8.328±2.711
LPS	0.600±0.047	2.410±0.825	90.39±2.11	9.008±2.129
Burn + LPS	0.252±0.077	2.784±0.957	90.88±1.03	8.866±1.027
p(LPS – burn+LPS)	<0.01	>0.05	>0.05	>0.05
HAES-LX 5 %	0.616±0.134	2.480±0.812	90.21±1.78	9.174±1.811
Burn + HAES-LX 5 %	0.320±0.047	2.512±0.406	91.82±1.36	7.862±1.336
p(HAES-LX5% – burn+HAES-LX 5%)	<0.01	>0.05	>0.05	>0.05
p(burn+0.9% NaCl – burn+LPS)	>0.05	<0.01	>0.05	>0.05
p(burn+0.9% NaCl – burn+HAES-LX 5%)	=0.060	<0.01	>0.05	>0.05
p(burn+ LPS – burn+HAES-LX 5%)	>0.05	>0.05	>0.05	>0.05

7 days after thermal skin burn on the background of the use of solutions of LPS or HAES-LX 5 % significantly lower ($p < 0.01$ in both cases) values of the S-phase, compared with similar groups without burn damage, and significant or trends of differences between indicators G0G1, G2+M and SUB-G0G1 were not detected (table 3) No significant or trend differences were found when comparing the respective parameters of the thyroid cell cycle between groups with skin burns after 7 days using 0.9 % solution of NaCl, LPS and HAES-LX 5 % (table 3).

Table 3

Indices of the cell cycle in rat thyroid cells 7 days after skin burn when using infusion therapy according to flow DNA cytometry ($M \pm \sigma$).

Group	Indices of the cell cycle (%)			
	S	SUB-G0G1	G0G1	G2 + M
0,9 % NaCl	0.650±0.139	2.632±0.724	90.90±2.17	8.448±2.113
Burn + 0,9 % NaCl	0.350±0.088	3.994±1.204	88.70±3.13	10.95±3.14
LPS	0.672±0.133	2.510±1.006	91.06±1.68	8.276±1.647
Burn + LPS	0.342±0.036	2.888±0.523	91.51±1.81	8.146±1.814
p(LPS – burn+LPS)	<0.01	>0.05	>0.05	>0.05
HAES-LX 5 %	0.592±0.076	2.662±0.711	90.32±1.78	9.084±1.757
LPS + HAES-LX 5 %	0.384±0.072	2.900±1.043	91.17±1.47	8.446±1.474
p(HAES-LX5% – burn+HAES-LX 5%)	<0.01	>0.05	>0.05	>0.05
p(burn+0.9% NaCl – burn + LPS)	>0.05	>0.05	>0.05	>0.05
p(burn+0.9% NaCl – burn+HAES-LX 5%)	>0.05	>0.05	>0.05	>0.05
p(burn+ LPS – burn+HAES-LX 5%)	>0.05	>0.05	>0.05	>0.05

Against the background of the use of the first seven days of LPS or HAES-LX 5 % solutions 14 days after skin burn in rats, only significantly lower ($p < 0.01$ in both cases) S-phase values were found, compared with similar groups without burn damage, and significant or trends in discrepancies between G0G1, G2+M and SUB-G0G1 were not detected (table 4). When comparing the respective parameters of the cell cycle of thyroid cells 14 days after skin burns between groups of 0.9 % solution of NaCl and LPS or HAES-LX 5 % it was found: a slight trend ($p = 0.076$) of lower values of the interval SUB-G0G1 when using LPS compared to 0.9 % NaCl solution; significantly higher ($p < 0.01$) values of the number of cells in the S-phase, a slight trend ($p = 0.076$) of a larger value of the G0G1 phase and a smaller ($p = 0.076$) value of the G2+M phase when using HAES-LX 5 % compared to 0.9 % solution NaCl; the tendency ($p = 0.060$) of a larger number of cells in the S-phase when using HAES-LX 5 % compared with LPS (table 4).

Indices of the cell cycle in rat thyroid cells at 14, 21 and 30 days after skin burns when using infusion therapy according to flow DNA cytometry (M±σ).

Group	Indices of the cell cycle (%)			
	S	SUB-G0G1	G0G1	G2 + M
14 day				
0,9 % NaCl	0.562±0.153	2.304±0.835	91.29±1.49	8.146±1.520
Burn + 0,9 % NaCl	0.322±0.043	3.664±0.239	89.15±3.56	10.53±3.54
LPS	0.658±0.168	2.812±0.772	90.54±1.69	8.798±1.736
Burn + LPS	0.354±0.042	2.864±0.603	91.31±1.17	8.334±1.164
p(LPS – burn+LPS)	<0.01	>0.05	>0.05	>0.05
HAES-LX 5 %	0.586±0.146	2.326±1.096	91.24±1.85	8.176±1.881
Burn + HAES-LX 5 %	0.394±0.021	3.104±0.893	92.00±1.22	7.602±1.226
p(HAES-LX5% – burn+HAES-LX 5%)	<0.01	>0.05	>0.05	>0.05
p(burn+0.9% NaCl – burn + LPS)	>0.05	=0.076	>0.05	>0.05
p(burn+0.9% NaCl – burn+HAES-LX 5%)	<0.05	>0.05	=0.076	=0.076
p(burn + LPS – burn+HAES-LX 5%)	=0.060	>0.05	>0.05	>0.05
21 day				
0,9 % NaCl	0.522±0.075	2.622±0.677	90.60±2.48	8.986±2.370
Burn + 0,9 % NaCl	0.364±0.092	3.250±0.755	87.98±3.30	11.66±3.27
LPS	0.556±0.166	2.742±0.513	91.88±1.74	7.558±1.595
Burn + LPS	0.392±0.067	2.616±0.984	89.13±3.29	10.48±3.28
p(LPS – burn+LPS)	=0.076	>0.05	>0.05	>0.05
HAES-LX 5 %	0.594±0.157	2.266±0.623	90.60±2.11	8.804±2.187
Burn + HAES-LX 5 %	0.444±0.063	3.844±0.372	91.01±1.11	8.544±1.119
p(HAES-LX5% – burn+HAES-LX 5%)	=0.076	<0.01	>0.05	>0.05
p(burn+0.9% NaCl – burn + LPS)	>0.05	>0.05	>0.05	>0.05
p(burn+0.9% NaCl – burn+HAES-LX 5%)	>0.05	>0.05	>0.05	>0.05
p(burn + LPS – burn+HAES-LX 5%)	>0.05	=0.060	>0.05	>0.05
30 day				
0,9 % NaCl	0.592±0.193	2.630±0.717	91.16±1.82	8.252±1.851
Burn + 0,9 % NaCl	0.408±0.063	2.900±1.078	83.11±2.14	16.50±2.18
LPS	0.590±0.216	2.600±1.013	90.84±1.94	8.570±1.767
Burn + LPS	0.444±0.052	3.624±0.487	90.84±4.33	8.716±4.319
p(LPS – burn+ LPS)	>0.05	<0.05	>0.05	>0.05
HAES-LX 5 %	0.582±0.133	2.232±0.417	91.31±2.49	8.110±2.409
Burn + HAES-LX 5 %	0.478±0.041	3.244±0.710	88.85±3.70	10.67±3.72
p(HAES-LX5% – burn+HAES-LX 5%)	>0.05	<0.05	>0.05	>0.05
p(burn+0.9% NaCl – burn+LPS)	>0.05	>0.05	<0.05	<0.05
p(burn+0.9% NaCl – burn+HAES-LX 5%)	>0.05	>0.05	<0.05	<0.05
p(burn + LPS – burn+HAES-LX 5%)	>0.05	>0.05	>0.05	>0.05

21 days after thermal burn of the skin on the background of the first seven days of LPS solution, only a slight tendency ($p=0.076$) to lower values of the number of cells in the S-phase compared with the same group without burns (table 4). At this time after the burn on the background of the first seven days of HAES-LX 5 % solution also found a slight tendency ($p=0.076$) to lower values of the number of cells in the S-phase and significantly ($p<0.01$) higher values of DNA fragmentation in the range SUB-G0G1 compared with a similar group of animals without burns (table. 4).

After 30 days in animals after skin burns on the background of the first seven days of LPS or HAES-LX 5 % solutions were found significantly ($p<0.05$ in both cases) higher values of DNA fragmentation in the range of SUB-G0G1 compared to similar groups without burns (table 4). When comparing the corresponding parameters of the cell cycle of thyroid cells 30 days after skin burns between groups of 0.9 % NaCl solution and LPS or HAES-LX 5 % found significantly higher ($p<0.05$ in both cases) values of the G0G1 phase, as well as smaller ($p<0.05$ in both cases) value of the phase G2+M when using LPS or HAES-LX 5 % compared with 0.9 % NaCl solution (table 4).

Summing up the results, it should be noted that the most pronounced disorders of the cell cycle were observed 3 days after thermal damage to the skin, although the first signs of these disorders in the form of a significant decrease in DNA synthesis ($p<0.01$) were observed after 1 day. In our opinion, this indicates a powerful damage to the thyroid gland caused by exogenous factors and activation of the endocrine system of the whole organism, which was mainly realized at the level of inhibition of DNA synthesis [4]. However, we cannot deny the possible protective nature of this phenomenon, because inhibition of cell division reduces

further cell destruction [2]. Our data support the assumption of the occurrence of 3 days after the burn of complex thyroid damage with the development of a deficit of energy and repair processes, which was also found by other researchers at similar times [2, 4, 14]. It is likely that in this period occurs peak deepening of cell damage that occurred at the time of thermal injury and their mass death in the form of apoptosis, which was established in similar clinical and laboratory studies [9-11] and begins to actively implement the protective effect of the studied hyperosmolar solutions. Note that the use of HAES-LX 5 % or LPS solutions, compared with 0.9 % NaCl solution has a more significant protective effect on the activation of apoptosis (interval SUB-G0G1) in thyroid damage on the background of thermal skin burns.

In favor of this hypothesis indicate the changes detected in the future, 7 days after the burn – when we found a violation of the cell cycle in the form of a decrease in S-phase, stored in the groups of burns + 0.9 % NaCl solution, burn + LPS and burn + HAES-LX 5 %, although other indices of the cell cycle of thyroid cells did not differ significantly from similar indices of the control groups. In our opinion, this indicates the need for longer infusion therapy with hyperosmolar solutions, which corresponds to the general trend of prolongation of infusion therapy against the background of thermal damage [5].

In the subsequent terms of research, the long-term negative effect of thermal damage of skin on indices of a cellular cycle of cells of a thyroid gland by a DNA cytometry was established. In our opinion, this indicates the existence of a long-term negative impact of the effects of thermal skin burns on the cell cycle of thyroid cells, which may cause long-term manifestations of BD, which are found in other organs and systems [3]. The obtained results are consistent with the results of studies of long-term effects of various toxic factors on the thyroid gland [1, 2]. This assumption is supported by the absence of changes in G0G1 phase and, accordingly, G2+M phase in groups using HAES-LX 5 % or LPS after 30 days, while maintaining larger ($p < 0.05$) values of the interval SUB-G0G1 compared to the same indices of groups without burn.

Thus, compared with 0.9 % NaCl solution, the use of LPS and HAES-LX 5 % more effectively corrected the violation of cell division, starting 3 days after the burn skin injury, which, in our opinion, indicates a more significant renewal of thyroid cells gland, which occurs in this organ by apoptosis [1].

A further perspective of the study is to study the different modes of administration of hyperosmolar solutions on the indices of the cell cycle of thyroid cells.

Conclusion

After 1, 3, 7 and 14 days after thermal trauma of the skin and the use of hyperosmolar solutions of LPS or HAES-LX 5 %, only lower ($p < 0.05-0.01$) values of S-phase values were found in comparison with the indices of groups without burns. 21 days after thermal damage to the skin in the group with infusion HAES-LX 5 %, the interval SUB-G0G1 is significantly higher ($p < 0.01$) compared with the same control group. After 30 days of thermal skin injury in the groups with prior administration of HAES-LX 5 % and LPS solutions, the value of SUB-G0G1 is significantly higher ($p < 0.05$) than in groups without skin burns.

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Реферати

**ПОКАЗНИКИ КЛІТИННОГО ЦИКЛУ
В ЩИТОПОДІБНІЙ ЗАЛОЗІ ПІСЛЯ
ТЕРМІЧНОГО ОПІКУ ШКІРИ ПРИ
ЗАСТОСУВАННІ РОЗЧИНІВ ЛАКТОПРОТЕІНУ
З СОРБИТОЛОМ АБО HAES-LX 5 %**

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Черешнюк І.Л., Лисенко Д.А.**

Вміст ДНК в ядрах клітин щитоподібної залози 90 білих шурів-самців на фоні опіку шкіри 2-3 ступеня (із площею ураження 21-23 % поверхні тіла) і введення розчинів лактопротеїну з сорбітолом або HAES-LX 5 % визначали методом проточної цитометрії. Через 1, 3, 7 та 14 діб після термічної травми шкіри і застосування лактопротеїну з сорбітолом або HAES-LX 5 % встановлено лише менші значення показників S-фази у порівнянні із показниками груп без опіку. Через 21 добу після термічного uszkodження шкіри в групі з інфузією HAES-LX 5 % показник інтервалу SUB-G0G1 суттєво більший порівняно з аналогічним показником контрольної групи. Через 30 діб в групах з попереднім введенням розчинів HAES-LX 5 % та лактопротеїну з сорбітолом величина показнику SUB-G0G1 значно більша від аналогічного в групах без опіку шкіри.

Ключові слова: щитоподібна залоза, термічний опік шкіри, ДНК-цитометрія, HAES-LX 5 %, лактопротеїн із сорбітолом.

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**ПОКАЗАТЕЛИ КЛЕТОЧНОГО ЦИКЛА
В ЩИТОВИДНІЙ ЖЕЛЕЗЕ ПОСЛЕ
ТЕРМИЧЕСКОГО ОЖОГА КОЖИ ПРИ
ПРИМЕНЕНИИ РАСТВОРОВ ЛАКТОПРОТЕИНА
С СОРБИТОЛОМ ИЛИ HAES-LX 5 %**

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Черешнюк И.Л., Лысенко Д.А.**

Содержание ДНК в ядрах клеток щитовидной железы 90 белых крыс-самцов на фоне ожога кожи 2-3 степени (с площадью поражения 21-23 % поверхности тела) и введение растворов лактопротеина с сорбитолом или HAES-LX 5 % определяли методом проточной цитометрии. Через 1, 3, 7 и 14 суток после термической травмы кожи и применения лактопротеина с сорбитолом или HAES-LX 5 % установлено только меньшие значения показателей S-фазы по сравнению с показателями групп без ожога. Через 21 день после термического повреждения кожи в группе с инфузией HAES-LX 5 % показатель интервала SUB-G0G1 существенно больше по сравнению с аналогичным показателем контрольной группы. Через 30 суток в группах с предварительным введением растворов HAES-LX 5 % и лактопротеина с сорбитолом величина показателя SUB-G0G1 значительно больше аналогичного в группах без ожога кожи.

Ключевые слова: щитовидная железа, термический ожог кожи, ДНК-цитометрия, HAES-LX 5 %, лактопротеин с сорбитолом.

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**MORPHOGENESIS OF RAT'S THYROID GLAND IN PREWEANING PERIOD AFTER
PRENATAL INFLUENCE OF STAPHYLOCOCCAL TOXOID**

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The results were obtained about morphogenesis of rat's thyroid after intrauterine antigenic action of staphylococcal toxoid. Prenatal influence of staphylococcal toxoid led to the formation of a more pronounced structure of the parenchyma and stroma, but they showed signs of functional immaturity, which led to the presence of a morphological picture of hypothyroidism after birth (rats 1-7 days of postnatal ontogeny). With the beginning of the middle sucking period (7-21 days of life) there is a lymphoid infiltration in separate sites of a thyroid gland, processes of reorganization of the synthetic device and resorption of colloid, so functional "maturation" of already morphologically formed structures begins. Such abrupt changes in the thyroid gland of experimental animals are due to systemic prenatal antigenic influence on the body as a whole and is adaptive-compensatory in nature.

Keywords: thyroid gland, antigen, staphylococcal toxoid, morphogenesis, experiment.

The work is a fragment of the research project "Immunomorphological features of internal organs under the effect of endogenous and exogenous factors on the body", state registration No. 0118U004250.

The current stage of the morphology development is characterized by increasing interest to the morphological and functional components of tissues, organs and their systems, that's why the issue of dynamic morphology is gaining more and more recognition. The thyroid gland (thyroid), is a heterogeneous tissue complex system [2, 6]. Structural and functional alteration of thyrocytes in various pathological conditions is a topical problem of biology and medicine, because thyroid hormones influence to the numerous processes of the vital activity in the body. Many authors note lability in morphology and functional activity of the thyroid gland in response to various aggressive factors of both exogenous and endogenous nature [2, 4, 6]. Antigenic influence at critical terms of ontogenesis can cause significant changes in the child's immune system. It is known that the entry of antigens into the fetus causes premature release of T-lymphocytes from the thymus and their migration to various organs. In these organs the tempo and terms change in formation of the basic structural components [1, 3, 5]. The appearance of more "aggressive" bacterial and viral pathogens led to a significant increase in the number of thyroid pathology [7, 8]. The thyroid gland, like every other organ, is characterized by a specific algorithm of functioning, which has the appropriate morphological design in the form of a hierarchy of cellular, tissue and organ units [2, 4, 6, 8].

The problem of infections caused by gram-positive microorganisms is growing steadily around the world. Staphylococci are the causative agents of much of the pneumonia, infections of the skin and soft tissues, bones and articulations, sepsis, etc. Staphylococcal infection poses the greatest threat to newborns and children in the first months of life. Therefore, in order to reduce the incidence among them, there are active immunization of pregnant women "at risk" with staphylococcal toxoid at 32, 34 and 36 weeks of pregnancy. Structural and functional changes, reactivity of structural and functional units, cellular and non-cellular components of the thyroid during postnatal ontogenesis in norm and after intrauterine action of staphylococcal toxoid have not been studied yet. Therefore, we consider the study relevant, given its practical significance and direction.

The purpose of the work was to study the features of rat thyroid gland's morphogenesis in suckling period at norm and after prenatal influence of staphylococcal toxoid.

Materials and methods. The material for the study was the thyroid gland of Wistar rats aged 1 to 21 days of postnatal development (108 animals), about 6 animals in each group. Three animal groups were studied on days 1, 3, 7, 11, 14, 21 after birth. Group I included intact animals (norm); group II was the control, animals which were injected intrauterine 0.9% NaCl solution; group III consisted of experimental animals injected with staphylococcal toxoid liquid purified adsorbed (10-14 units of binding in 1 ml, diluted 10 portions) intrauterine, on the 18th day of dated pregnancy by the method of Voloshin MA (Pat. 49377, Ukraine, 2010 and Pat. 63020, Ukraine, 2011). Injections of antigen or 0.9% NaCl solution for fetus were performed surgically during laparotomy, by intrauterine, transdermal subcutaneous ways at the dose of 0.05 ml to each fetus. The distribution of the material is presented in table 1.

Table 1

Distribution of material under study

Age groups of the suckling period	Day of a life	Intact group	Control group	Experimental group
Newborns (early suckling)	1	6	6	6
	3	6	6	6
	7	6	6	6
Suckling (middle suckling)	11	6	6	6
	14	6	6	6
	21	6	6	6
Total		36	36	36

The thyroid gland was fixed in a 10% solution of neutral buffered formalin during the day. The objects were filled into paraffin blocks by the conventional method. Histological sections 3-5 μ m thick were stained by hematoxylin-eosin, histochemically by PAS reaction, by Van Gizon staining, by azure-II-eosin. A set of morphometric studies was performed by microscope Carl Zeiss Primo Star equipped with the Axiocam digital microphoto attachment with using program complex Zeiss Zen 2011. The results were considered reliable at $p < 0.05$. To summarize the digital material, for processing of statistical material the standard software packages were used: Microsoft Office Excel and Statistica 10.0.

Results of the study and their discussion. In serial sections of the animals' thyroid in the intact and control groups from 1st day of life at early suckling period it was proved, that parenchyma is characterized by the presence of numerous globular clusters of thyrocytes - follicles without colloid, which are closely neighboring upon each other (fig. 1a).

Thyrocytes have cuboidal form, they are not yet clearly distinguished by apical and basal poles. The nuclei contain 1-3 nucleoli, which indicates a protein-synthetic activity. The basement membrane is not visualized, so these globular formations have the appearance of a chaotic cell mass, but not organized structures. On the 3rd day, single follicles occur with colloidal type of secretion, which are located mainly under the thyroid capsule (fig. 1.b).

In animals of early suckling period in intact and control groups the thyroid stroma is weakly expressed, the capillary network is not clearly visualized between the follicles, but the thyroid parenchyma is divided into lobes by layers of connective tissue and contains vessels and nerves.

The microscopic study of serial sections of a thyroid gland in experimental group (prenatally antigen-awarded animals) at the age of 1-7 days, attention is drawn by recalibration of arteries, expansion of capillaries, increase in a stromal component between follicles of colloidal type in the thyroid gland. During this period, colloidal follicles occur throughout the thyroid, but mostly under the capsule, in contrast to animals of the intact and control groups.

In animals of the intact and control groups on the 7th day, the ratio of follicles of different sizes (small: medium: large) is 3: 1: 0, and in antigen-premium - 1: 2: 1, respectively. In the ratio of tissue components a decrease was revealed in the specific area of the thyroid epithelium. This is due to the fact that the height of the cells of the follicular epithelium becomes smaller, the cubic and flat form of thyrocytes predominates, cylindrical cells are rare, mainly in adenomers of small size of the noncolloid type. An increase in the relative percentage of the area of the colloid due to an increase in the number of large and

medium-sized follicles, which contain dense colloid and desquamated cells (fig. 1b). Boundary vacuolation of the colloid is not detected.

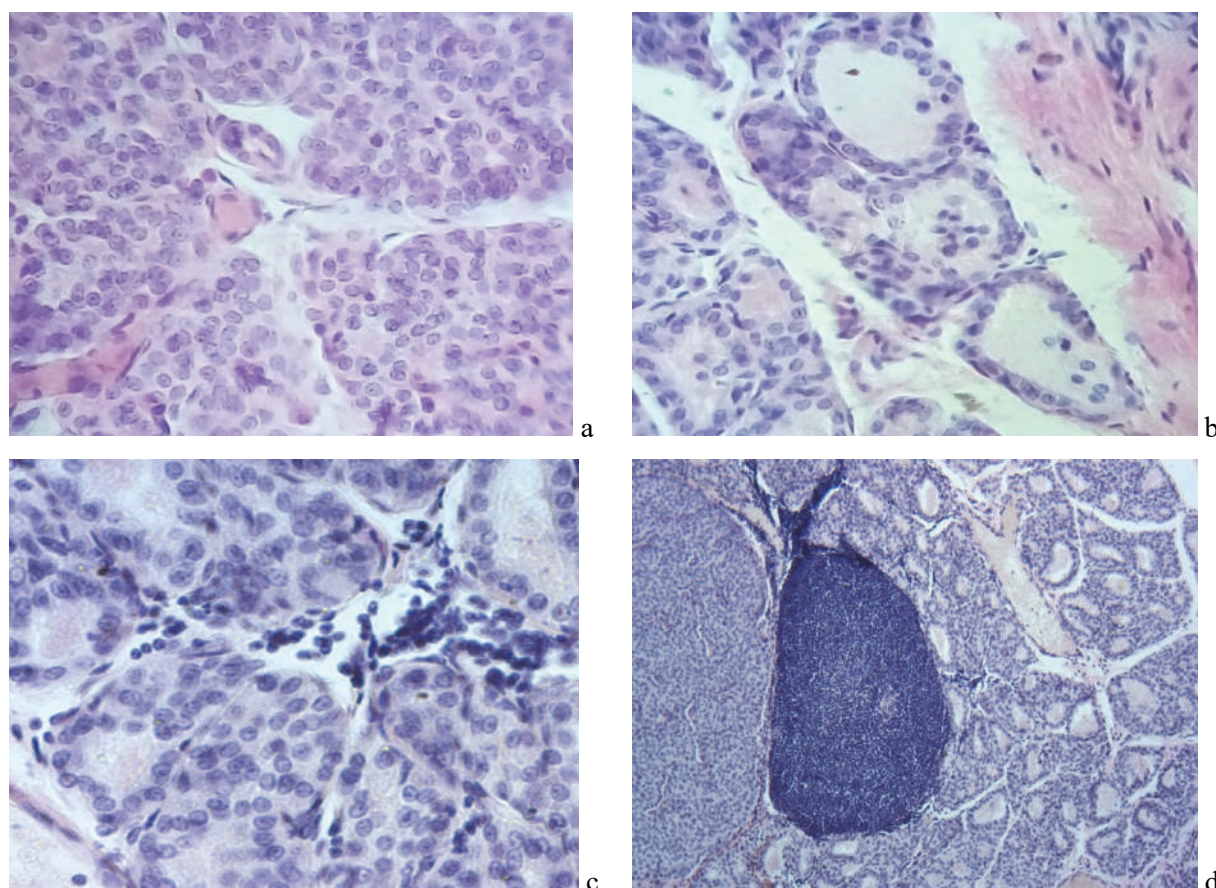


Fig. 1. Thyroid gland of suckling period rats on the 3rd day of life. a - intact group of animals; b - experimental group. $\times 600$. Lymphocytic infiltration in the thyroid gland of rats after prenatal exposure to staphylococcal toxoid. H&E stain. c - 7th day of life, $\times 600$. d - 21st day of life, $\times 150$.

In animals after intrauterine action of staphylococcal toxoid for 1-7 days, the average diameter of the nuclei is significantly reduced compared to the control group and is $0.25 \pm 0.01 \mu\text{m}$. There is a tendency to decrease the number of nucleoli in thyrocytes of large follicles and to increase in adenomers of medium and large diameters (tab. 2)

Table 2

Percentage of thyrocytes with different numbers of nucleoli in the follicles of suckling animals of control and experimental groups ($M \pm m$), $p < 0.05$

Group	Intact			Experimental		
	Numbers of nucleoli in thyrocytes					
	1	2	3 and more	1	2	3 and more
Large	22 \pm 2,03	30,7 \pm 1,05	49 \pm 1,03	19,7 \pm 0,04	32,7 \pm 3,02	47,7 \pm 3,04
Middle	22,7 \pm 4,05	33,7 \pm 3,01	44,3 \pm 2,03	29,7 \pm 2,05	23,7 \pm 3,04	46,7 \pm 2,01
Small	25 \pm 3,01	31,3 \pm 3,03	43,7 \pm 6,04	25,3 \pm 1,05	26,7 \pm 2,01	48 \pm 4,03

The decrease in the number of nucleoli in follicular thyrocytes indicates a decrease in the synthesis of ribosome subunits, and hence the gross synthesis of protein products. This is accompanied by a slowdown in the excretion of hormones into the bloodstream (absence of resorption vacuoles in the colloid). Stagnation of secretion in the follicle cavity can lead to stretching of its walls, causing signs of hypertrophy.

PAS reaction in the thyroid gland of antigen-premium animals show, that the colloid in large diameter follicles (++) has the most intense color. In medium and small adenomers, the reaction is less intense (+). The colloid of single follicles does not show PAS-positive properties. The cytoplasm of thyrocytes of follicles of different diameters is brightly colored over the entire area of the organ, but the intensity of the PAS reaction is more pronounced in prismatic and cubic thyrocytes, which correlates with manifestations of functional tension (synthesis and secretion of thyroid hormones).

Starting from the 7th day of life in antigen-premium animals in the subcapsular zone of the thyroid gland there are places of eviction of small and medium lymphocytes by diapedesis through the wall of the venules and their migration around the follicles (fig. 1c). Colloid-type adenomers in such locations on the 11th day already showed board vacuolation of the colloid (not a characteristic picture for the entire

parenchyma), cubic thyrocytes with 2-3 nucleoli. In the cells of the thyroid epithelium are often visible figures of mitosis. Most often, such diffuse clusters of lymphocytes were localized near the border of the thyroid gland with the parathyroid glands.

In the middle suckling period, the thyroid gland of animals of the intact and control groups was characterized by an increase in the number of colloid type follicles. Cuboidal thyrocytes was detected moderate board vacuolation of colloid, which indicates active hormone-producing activity.

In animals of the experimental group from the 7th to the 21st day of the postnatal period there was a more rapid rate of folliculogenesis and the development of connective tissue components of the stroma. Adenomers mostly of medium and large size with rapid board vacuolation of the colloid, lined with cubic thyroid epithelium. 2-4 nucleoli are present in the nuclei of thyrocytes. At the sites of lymphocyte migration into the connective tissue layers, near the venule wall, mast cells with metachromatic granules without signs of mass degranulation are detected. On the 14th day, a clearly formed, mostly single (lymphoid) nodule with lymphocytic cords (Fig. 1d) is visible, which consists mainly of small and medium lymphocytes, without a germinal center and a vaguely formed capsule. Desquamated cells of the thyroid epithelium are often visible in the colloid adenomers adjacent to the lymphoid nodule, while the shape of the follicles, the integrity of the epithelium and the basement membrane are not violated.

On the 21st day, in addition to diffuse lymphoid infiltration on the periphery of the thyroid lobes, the presence of a germinal center in the lymph node is noteworthy. The density of lymphocytes in the central zone of the nodule is lower than in the peripheral, among the lymphocytes of the central zone there are macrophages and plasma cells.

Thus, after the prenatal action of staphylococcal toxoid on the fetus, the rate of thyroid morphogenesis changes dramatically. This tendency of morphological changes of parenchymal organs is substantiated by a number of studies of other scientists [1, 3, 5, 7]. Thyroid folliculogenesis accelerates, but despite this, in the early dairy period on serial sections revealed morphological signs that indicate a decrease in the functional activity of the organ. Among them: an increase in the number of large and medium-sized follicles, a decrease in the height of the follicular epithelium, the lack of marginal vacuolation of the colloid. These data correlate with transformations at the cellular level, namely, there is a decrease in the number of nucleoli and a decrease in the concentration of RNA in the cytoplasm of thyrocytes, which indicates the suppression of synthetic processes occurring in the cell. With the advent of lymphocytic infiltration with the onset of the middle suckling period in the thyroid gland in large and medium follicles increases the height of the thyroid epithelium, the number of nucleoli in the nuclei of thyrocytes, increases regenerative-desquamation processes, begins resorption of colloid and active production of hormones in the blood by morphologically formed structures. Such abrupt changes in the thyroid gland of experimental animals are due to systemic prenatal antigenic influence of the body as a whole and has an adaptive-compensatory nature, both on the part of the immune system and the thyroid gland, because its hormones directly affect the body's development under endogenous and exogenous factors confirmed by other studies [2, 6, 8]. Thyroid transformation under prenatal antigenic influence on the fetus is an immunologically dependent dynamic process with sequential deployment and change of regular stages with the release of a polymorphoplastic variant of the thyroid gland in suckling rats, and coincides with the concept of studies of various organs under antigenic conditions. [1, 3, 5, 7].

Conclusions

Intrauterine injection of staphylococcal toxoid led to the formation of a more pronounced structure of both parenchymal and stromal elements, but with morphological signs of functional immaturity and at the early stages of development with a morphological picture of signs of the thyroid gland's hypofunction. Structural and functional changes of the thyroid gland in the experimental group may be associated with the indirect influence of lymphoid cells on the morphogenesis of the organ, which is the purpose of further study. The obtained data are important for pediatricians, endocrinologists, immunologists in explaining certain etiopathogenetic aspects of autoimmune thyroiditis in children.

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Реферати

**МОРФОГЕНЕЗ ЩИТОПОДІБНОЇ ЗАЛОЗИ ЩУРІВ
МОЛОЧНОГО ПЕРІОДУ
ПІСЛЯ ПРЕНАТАЛЬНОЇ ДІЇ
СТАФІЛОКОКОВОГО АНАТОКСИНУ**

Федосєєва О.В.

Одержано результати щодо темпів морфогенезу щитоподібної залози після внутрішньоплідної антигенної дії стафілококкового анатоксину. Пренатальне введення стафілококкового анатоксину призвело до формування більш вираженого структуроутворення елементів паренхіми і стромы, але вони мали ознаки функціональної незрілості, що призвело до наявності морфологічної картини гіпотиреозу вже після народження (щери 1-7 доби постнатального онтогенезу). З початком середнього молочного періоду (7-21 доба життя) з'являється лімфоцитарна інфільтрація в окремих ділянках щитоподібної залози, відбувається перебування синтетичного апарату та процесу резорбції колоїду, тобто починається функціональне «дозрівання» вже морфологічно сформованих структур. Такі скачкоподібні зміни в щитоподібній залозі експериментальних тварин обумовлені системним пренатальним антигенним навантаженням організму в цілому та носить пристосувально-компенсаторний характер.

Ключові слова: щитоподібна залоза, антиген, стафілококковий анатоксин, морфогенез, експеримент.

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**МОРФОГЕНЕЗ ЩИТОВИДНОЇ ЖЕЛЕЗЫ КРЫС
МОЛОЧНОГО ПЕРИОДА
ПОСЛЕ ПРЕНАТАЛЬНОГО ДЕЙСТВИЯ
СТАФИЛОКОККОВОГО АНАТОКСИНА**

Федосеева О.В.

Получены результаты о темпах морфогенеза щитовидной железы после внутриплодного антигенного воздействия стафилококкового анатоксина. Пренатальное введение стафилококкового анатоксина привело к формированию более выраженного структурообразования элементов паренхимы и стромы, которые имели признаки функциональной незрелости, что привело к наличию морфологической картины гипотиреоза уже после рождения (крысы 1-7 суток постнатального онтогенеза). С началом среднего молочного периода (7-21 сутки жизни) появляется лимфоцитарная инфильтрация в отдельных участках щитовидной железы, происходит перестройка синтетического аппарата и процесса резорбции коллоида, то есть начинается функциональное «созревание» уже морфологически сформированных структур. Такие скачкообразные изменения в щитовидной железе экспериментальных животных обусловлены системным пренатальным антигенным воздействием на организм в целом, и носит приспособительные-компенсаторный характер.

Ключевые слова: щитовидная железа, антиген, стафилококковий анатоксин, морфогенез, експеримент.

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**ULTRASTRUCTURAL ORGANIZATION FEATURES OF PERIODONTAL TISSUES AFTER
TWELVE WEEKS OF OPIOID INFLUENCE**

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In the experiment on white rats, ultrastructural studies of periodontal tissues were carried out against the background of twelve-weeks opioid action. Animals were subjected to intramuscular injections of an opioid analgesic with a gradual increase in the mean single dose every two weeks. The initial dose was 0.212 mg/kg, and within 11–12 weeks the dose was increased up to 0.3 mg/kg. The results of submicroscopic studies have revealed the progression of chronic sclerosing inflammation in the periodontium and the development of regenerative-plastic deficiency of epitheliocytes, endothelial cells and the periodontium structural components, which was caused by a long-term opioid action.

Key words: periodontium, rat, opioid, ultrastructure.

The work is a fragment of the research project “Morpho-functional features of organs in the pre – and postnatal periods of ontogenesis, under the influence of opioids, food supplements, reconstructive surgery and obesity”, state registration No. 0120U002129.

Periodontal diseases of a dystrophic-inflammatory nature are quite widespread in practically most age groups with more than 75% of the population affected worldwide, which is an important socio-medical problem [1, 3, 6, 12, 13]. In particular, a number of researchers consider generalized periodontitis a polyetiological disease with various mechanisms of pathogenesis [5, 6, 13]. In numerous disorders of metabolism that cause the development of morphological manifestations in periodontal tissues, complications occurring under the influence of narcotic intoxication play a significant role [4, 8]. It should be noted that the pathology of organs and tissues in the oral cavity of opioid-dependent persons has not been sufficiently

studied, which prompts the need for scientific research, including experimental models [9, 14, 15]. Due to the ethical aspects of human material use, animal models are widely applied in experimental medicine, so that the necessary information can be obtained and analysis of the tissues and organs structural organization can be performed with a gradual worsening of the pathological process [10, 7, 11, 14].

However, questions about the beginning of the pathomorphological changes development in the structural components of the periodontal complex, the dynamics of the opioid analgesic effect assuming the mean single therapeutic dose on the worsening of these changes in terms of hemodynamic disorders, as well as on the emergence of oral cavity pathogenic microflora against the background of the prolonged opioid action. In future, this will permit to solve the problem of figuring out the scheme for these changes correction at the early and late stages of chronic opioid exposure.

The purpose of the work was to study the features of periodontal tissues ultrastructural organization against the background of chronic exposure to opioid analgesics for twelve weeks under experimental conditions.

Materials and methods. The studies were performed on 22 white adult 4.5 – 7.5 months old Wistar male rats weighing 160 – 270 g. In the experiment, the animals were divided into two groups. The first group was intact rats (10). In the second group, animals (12) were daily administered nalbuphine opioid analgesic intramuscularly, belonging to the group of opiate receptor antagonist agonists, for 84 days. The starting dose was 0.212 mg/kg. During the 11 and 12 weeks, the mean single therapeutic dose was increased up to 0.3 mg/kg, taking into account the mean weight of the study group. Controls were 3 rats, intramuscularly injected with normal saline. The experimental animals were kept under vivarium conditions and the material was collected according to generally accepted rules. Before sampling, the animals were euthanized by intraperitoneal administration of sodium thiopental (25 mg/kg). For ultrastructural examination, pieces of soft periodontal tissue were used in the area of the maxillary and mandibular gingival papilla. The obtained tissue fragments were immediately placed for fixation into 2.5% solution of glutaraldehyde and into 1% solution of osmium tetroxide in phosphate buffer with pH 7.2 – 7.4. Subsequently, dehydration of tissue fragments in alcohols and propylene oxide was carried out and the samples were embedded into a mixture of epoxy resins with araldite [2]. Ultra-thin sections were made using the UMT3m ultramicrotome, followed by contrasting with uranyl acetate and lead citrate and studied in detail with PEM-100-01 electron microscope.

Results of the study and their discussion. Electron microscopic studies have shown that in the control group rats, the ultrastructural organization of periodontal tissues was preserved. The multilayered flat epithelium of the gingival mucosa had the usual layered structure of cells, nuclei were round shaped, nucleoli were dense, organelles were present. The periodontium components were clearly structured, intercellular substance interlayers, collagen fibers, fibroblasts, fibrocytes were visualized. Microcirculatory bed vessels had a well-defined structure of the endothelium and the basement membrane. Submicroscopic examination of the animal gums mucous membrane with long-term administration of opioid analgesics for twelve weeks revealed profound changes in all its cellular components.

In the basal layer of the epithelium in the spare part of gums, most of the epitheliocyte nuclei have uneven contours, karyolemma forms deep invaginations. Karyoplasm of electron light, nucleoli with segregated granular and fibrillar components. Intercellular contacts are damaged, reduced in some areas. Intercellular spaces are unevenly contoured and mostly enlarged, which is a sign of spongiosis. Cytoplasm of the spinous layer epitheliocytes includes diffusely arranged thickened bundles of tonofilaments. In the perinuclear zone, electron-lucent anhistic structures with formation of vacuole-like structures are present, indicating the development of partial necrosis. The karyolemma forms invaginations, nucleoli are segregated (fig. 1). In the cytoplasm of the granular layer epitheliocytes, many electron-dense keratogialin inclusions of different sizes are found.

Most nuclei of gingival crest epitheliocytes have uneven contours, the karyolemma forms invaginations. The karyoplasm is electron-lucent, nucleoli are absent. In the basal layer cells cytoplasm, most organelles are damaged. Mitochondria have reduced cristas, diffusely vacuolated. There are few tonofilaments, they are either fragmented or lysed. Intercellular contacts are indistinct, reduced; there are also unevenly enlarged intercellular spaces. In the spinous layer, the cytoplasm of the epitheliocytes includes many osmiophilic, irregularly shaped inclusions that were formed due to the increased keratinization.

In the attached part of the gums, significant changes in epitheliocytes, apoptosis phenomena, were found. In the nuclei there are signs of karyorexis and karyolysis, karyolemma is osmiophilic. Characteristic is the of subplasmolemmotic vacuolation of the cytoplasm and large-sized non-structural osmiophilic areas present in the epitheliocytes. Lysis and fragmentation of the tonofilaments, as well as the organelles destruction are caused by the progression of necrotic changes in the cell (fig. 2).

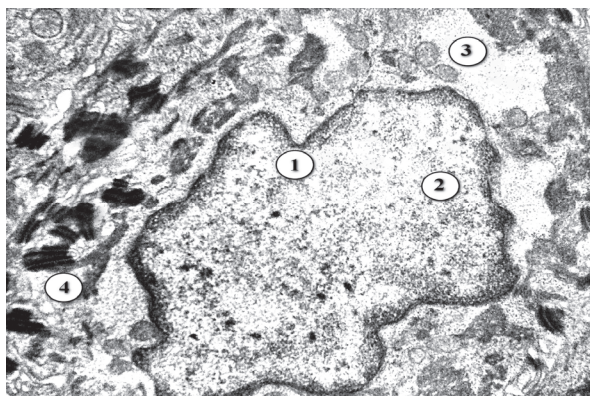


Fig. 1. The epitheliocyte ultrastructure in the epithelium spinous layer of the rat gingiva spare part after twelve weeks of opioid administration. 1 –karyolemma invaginations, 2 – nucleoli segregation, 3 – vacuole-like structures, 4 – tonofilament bundles. Electronogram. Approx. 12,000.

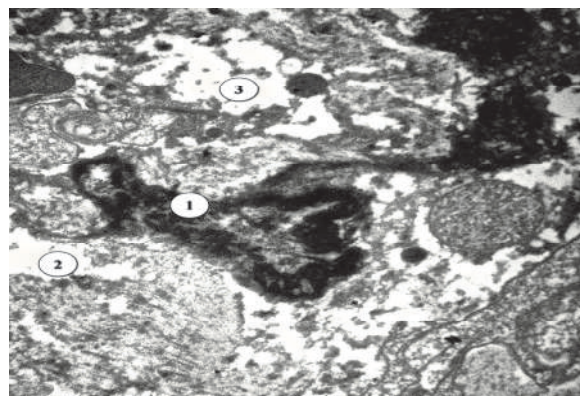


Fig. 2. Epithelium of the attached part of the rat gums after twelve weeks of opioid administration. 1 – karyolysis, 2 – vacuolation of the perinuclear zone, 3 – necrotic changes in the cytoplasm. Electronogram. Approx. 14,000.

Electron microscopic studies have also revealed pronounced changes in the structural components of the periodontium. The ultrastructure of fibroblasts, which showed signs of destruction in the nuclei, invagination of the karyolemma, is significantly disturbed. There are few organelles in the cytoplasm, they are destructively altered. Significant destruction of collagen fibers, their fragmentation and destruction have been found. There is a pronounced edema of the amorphous component of the intercellular substance of the connective tissue. The structure of fibrocytes is significantly altered, macrophages are activated and there are degranulated tissue basophils.

The performed ultrastructural studies have established profound changes in the vessels of the hemomicrocirculatory bed. During this period of experiment, the lumen of the blood capillaries is filled with blood cells, mainly erythrocytes, and there is a sludge effect. The of endotheliocyte nuclei are destructively altered, they are small, their pycnosis is present, with predominance of heterochromatin in the karyoplasm. The karyolemma produces deep invaginations. In the cytoplasm of endothelial cells, damaged organelles, a small number of pinocytotic vesicles were found. Sclerotic changes progress, as evidenced by the presence of collagen fibers in the perivascular space. The basement membrane is not distinctly contoured, it is thickened in some areas.

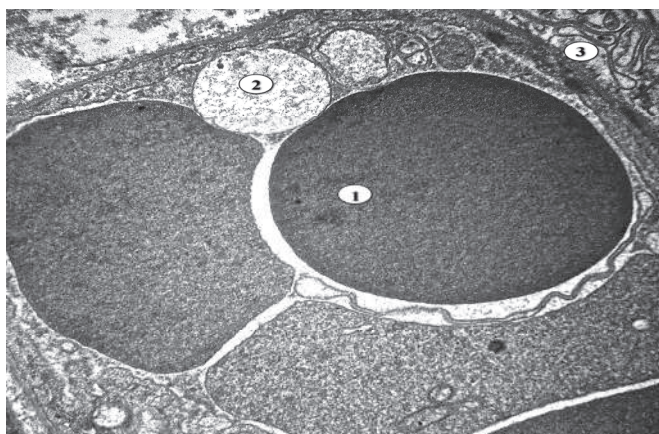


Fig. 3. Ultrastructure of the rat gingival mucosa venule after twelve weeks of opioid administration. 1 – wide lumen with erythrocytes, 2 – vacuole-like structures in the cytoplasm, 3 – bundles of tonofilaments. Approx. 9000.

Submicroscopically, dilated venule lumens with phenomena of congestion are observed in the late experimental period. In many endothelial cells, the nuclei are significantly altered, and the karyoplasm is osmophilic. The characteristic feature is numerous protrusions and focal lamination of the endothelial cell surface. In the cytoplasm, organelles are destructively altered, vacuole-like formations are of different size, phenomena of mitochondrial vacuolation are found, which is the first sign of cell autolysis. Perivascular spaces are significantly enlarged, the basement membrane is not distinctly contoured, and it is considerably thickened in some areas (fig. 3).

The experimental modeling results of long-term opioid exposure in rats correlate with the data of ultrastructural study in patients with gingivitis and generalized periodontitis [1, 4]. Twelve-weeks exposure to the opioid mediator revealed submicroscopically profound changes in the cellular components of the gingival epithelium, the periodontium connective tissue, and the components of the periodontium hemomicrocirculatory bed. With a long-term opioid effect, apoptosis phenomena occur, necrotic changes progress, vacuolation of the perinuclear zone is observed, indicating evidence of partial necrosis.

The obtained results also confirm the data on lesions of the hemomicrocirculatory bed components in the periodontium and accordingly the development of tissue ischemia and hypoxia, which is also one of the dominant signs in the periodontitis pathogenesis [5]. Since there are no results of studies on the periodontal cell components submicroscopic changes in dynamics at the late stages of experimental opioid exposure, it was not possible to compare them with the data of other researchers.

Conclusion

The performed ultrastructural study of soft periodontal tissues revealed the progression of chronic sclerosing inflammation and the development of regenerative-plastic deficiency of epitheliocytes, endotheliocytes and the periodontium structural components due to long-term opioid action.

Prospects for further research are to carry out a comparative analysis of pathomorphological changes in the periodontium at different times of opioid exposure and to develop a scheme of probable corrective effects.

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Реферати

**ОСОБЛИВОСТІ УЛЬТРАСТРУКТУРНОЇ
ОРГАНІЗАЦІЇ ТКАНИН ПАРОДОНТА
ЧЕРЕЗ ДВАНАДЦЯТЬ ТИЖНІВ ОПІОЇДНОГО
ВПЛИВУ**

Фік В.Б., Пальтов Є.В., Кривко Ю.Я.

В експерименті проведено ультраструктурні дослідження пародонту при дії опіоїдів протягом дванадцяти тижнів. Тваринам проводили внутрішньом'язові ін'єкції опіоїдного анальгетика з підвищенням разової дози кожні 2 тижні. Початкова доза – 0,212 мг/кг, протягом 11 – 12 тижнів збільшили до 0,3 мг/кг. Одержані результати субмікроскопічних досліджень встановили прогресування хронічного склерозуючого запального процесу в пародонті і розвиток регенераторно-пластичної недостатності епітеліоцитів, ендотеліоцитів і структурних складових пародонту, що обумовлено тривалою дією опіоїда.

Ключові слова: пародонт, щур, опіоїд, ультраструктура.

Стаття надійшла 28.08.2019 р.

**ОСОБЕННОСТИ УЛЬТРАСТРУКТУРНОЙ
ОРГАНИЗАЦИИ ТКАНЕЙ ПАРОДОНТА
ЧЕРЕЗ ДВАНАДЦАТЬ НЕДЕЛЬ ОПИОИДНОГО
ВЛИЯНИЯ**

Фик В.Б., Пальтов Е.В., Кривко Ю.Я.

В эксперименте проведены ультраструктурные исследования пародонта при действия опииодов на протяжении двенадцати недель. Животным проводили внутримышечные инъекции опиоидного анальгетика с повышением разовой дозы каждые 2 недели. Начальная доза – 0,212 мг/кг, в течение 11 – 12 недель увеличили до 0,3 мг/кг. Полученные результаты субмикроскопических исследований установили прогрессирование хронического склерозирующего воспалительного процесса в пародонте и развитие регенераторно-пластической недостаточности эпителиоцитов, эндотелиоцитов и структурных составляющих пародонта, что обусловлено длительной действием опиоида.

Ключевые слова: пародонт, крыса, опиоид, ультраструктура

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THE ROLE OF CHRONIC GASTRITIS AMONG PRECANCEROUS DISEASES OF THE STOMACH

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The purpose of the study is to statistically analyze the condition of the gastric mucosa affected by *Helicobacter pylori* in young people engaged in sports. Studies of chronic gastritis type B in student volunteers involved in sports. In 92% of cases, chronic gastritis were *Helicobacter pylori*-associated. Between the degree of contamination of the mucous membrane of *Helicobacter pylori* and the degree of leukocyte infiltration of the mucous membrane, the Pearson correlation coefficient r_{xy} is 0.935, the correlation is very strong, the determination coefficient is $D=r_{xy}^2=0.874$, the critical value of the correlation coefficient with a probability of 0.95–0.2732, the critical value of the correlation coefficient with a probability of 0.99–0.3511, comparing the correlation coefficient r_{xy} with a critical r_{cr} value for a significance of 0.95– $r_{xy}>r_{cr}$, comparing the correlation coefficient r_{xy} with a critical r_{cr} value for a significance of 0.99– $r_{xy}>r_{cr}$ to the covariance coefficient is 521.641, this makes it possible to conclude a statistically significant dependence with a probability of 0.99. Thus, chronic atrophic *Helicobacter pylori*-associated gastritis is a common disease of young people engaged in sports, and is central among precancerous diseases of the stomach.

Key words: *Helicobacter pylori*, gastritis, leukocyte infiltration, gastric mucosa

The work is a fragment of the research project "Realization of health savings technologies in physical education in the conditions of European integration of Ukraine", state registration No. 0117U003236.

Recently, diseases of the digestive system occupy a leading place not only in Ukraine, but throughout the world (70%). Sports are often accompanied by the risk of chronic gastritis. It should be emphasized that excessive and irrational training loads can be one of the important factors in the occurrence of chronic gastritis, especially if they are combined with a violation of diet. The most common disease of gastroenterology is chronic gastritis. It takes the second place in athletes after cardiovascular diseases. Chronic gastritis can occur: type A (autoimmune), type B (bacterial) and type C (chemical reactive reflux gastritis) [12]. Chronic type B gastritis is an infectious inflammation of the gastric mucosa, the etiological factor of which is *Helicobacter pylori*. The latter is transmitted by the alimentary route and is present in 6 out of 10 people. Many people are infected with it since childhood [2]. Its pathogenic effect is the partial neutralization of the acidic environment of the stomach, destruction of the integumentary epithelium and the epithelium of its glands [15]. The leading factor in chronic gastritis is heredity, and the genetic tendency to the disease itself [5].

Athletes are quite vulnerable to this disease, because they are constantly under the influence of endogenous and exogenous factors that contribute to the defeat of *Helicobacter pylori* [9]. These include: violation of the principle of rational nutrition (irregular and large intervals between meals, especially during competitions, bad habits in the form of overeating, alcohol consumption, smoking, prolonged use of drugs, the presence of food additives in food, poor environmental situation, poor quality food) [7].

There is the problem of food allergies (type A gastritis is autoimmune), which is characterized by a change in the body's response to protein intake, the immune system takes for an antigen. An example of such a product can be meat, fish, eggs, which are integral components of sports nutrition [8]. Daily athlete's body is subject to great physical exertion; it may be one of the factors of gastritis. Excessive physical exertion inhibits the functioning of the stomach and leads to secretory insufficiency. Type C gastritis is of a chemical nature and may be caused by the use of certain non-steroidal anti-inflammatory drugs [11]. Thus caused, duodenogastric reflux leads to the defeat of the gastric mucosa, mainly antrum, bile acids, their salts, pancreatic enzymes, lysolecithin and other components of the duodenum [1].

Despite frequent medical examinations of an athlete, it is difficult to detect chronic gastritis in him. Athletes often hide the symptoms of chronic gastritis, enduring even pain, is one of its symptoms [10]. Most often, a person feels discomfort in the vertex or pylori-duodenal region, nausea after eating, heartburn, intestinal upset, existing yellow-white plaque on the tongue [6]. Diagnosis is carried out in the form of gastroscopy, gastro biopsy [13].

Elimination of chronic gastritis in athletes is carried out by clinical nutrition, vitaminization, a local effect on the gastric mucosa. It is recommended to refuse training.

The purpose of the study was to provide a statistical analysis of the state of the gastric mucosa of the affected *Helicobacter pylori* in young people involved in sports.

Materials and methods. The work put the results of the study of chronic gastritis of type B in students of our university volunteers. The study was attended by students of 1–4 courses aged 17 to 25 years, only 25 people. Among the latter were 13 men and 12 women. In them, by agreement, gastrobiopsies were taken in the endoscopy room of the regional hospital for further study. Ethics and bioethics were respected.

Ethics Commission of the Petro Mohyla Black Sea National University noted that the study was carried out without violations by the students studied. The latter were fully aware of the methods and scope of the study. The study has been conducted voluntarily and anonymously in accordance with the requirements and standards, model regulations on ethics issues of the Ministry of Health of Ukraine No. 690 of September 23, 2009.

The colonization of *Helicobacter pylori* infection to the condition of the gastric mucosa was studied on semi-thin sections made of epoxy blocks (EPON 812). Gastrobiopsies of the gastric mucosa for the study were taken from the pyloric region of the lesser curvature and body of the stomach.

The fixative was a 10% neutral formalin solution or 4% cold glutaraldehyde solution in phosphate buffer at pH 7.4. The quality assessment of the obtained samples was carried out using a stereoscopic microscope. After a leak in the epoxy resin, the tissue blocks were placed in a capsule filled with resin, where the material was polymerized gradually at a temperature of 35°, 45° and 60°C - 24 hours each.

With paraffin or epoxy units of different topografoanatomic parts of the gastric mucosa in a microtome MPS-2 received slices, which were placed in a tray for slices and stained with haematoxylin and eosin and toluidine blue (to *Helicobacter infection pylori* study) by conventional schemes and placed in Canada balsam.

In the remaining stained sections, except for the epithelial components, the dye allows to detect the bacteria *Helicobacter pylori*.

When establishing the forms of chronic gastritis, classification of chronic gastritis, adopted at the 9th International Congress of Gastroenterologists in Sydney (1990). The following morphological forms of chronic gastritis were distinguished:

- 1) by etiology: an autoimmune associated with pyloric helicobacterium (HP); special form (eosinophilic, granulomatous);
- 3) according to the degree of morphological signs: no changes; flat erosive; atrophic; hyperplastic;
- 4) the nature of gastric secretion: with preserved or increased secretion; with secretory insufficiency.

To quantify the degree of contamination of the gastric mucosa of *Helicobacter pylori*, bacteria were counted in a field of view $\times 600$. There are weak (up to 20 bacteria), moderate (up to 50 bacteria) and high (more than 50 bacteria) degree of contamination. In this field of view, a semi-quantitative assessment of the degree of leukocyte infiltration of the gastric mucosa was also performed. When rare disseminated polymorphonuclear leukocytes were found in inflammatory infiltrates and in the layer of pathogenic epithelium, the degree of leukocyte infiltration was assessed as poorly expressed and, in the form of fields, as expressed.

The results obtained from morphometric studies were statistically processed using standard methods of variation statistics. The significance of differences in the average compared indicators was evaluated by the criteria (t) of the Student. The difference between comparable values was considered significant if the permissible error (p) was less than 0.05.

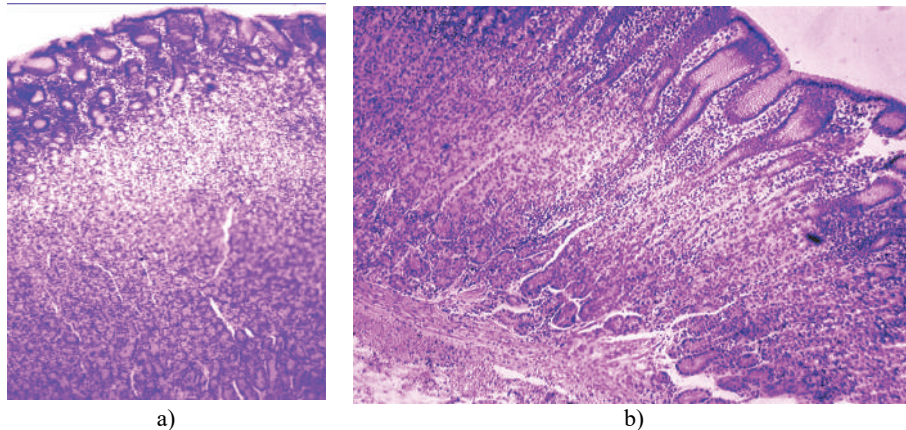


Fig.1: a) Superficial gastritis. Coloring hematoxylin-eosin. Mag. 180; b) Initial atrophic gastritis. Inflammatory infiltration is common below the foveolar layer. Coloring hematoxylin-eosin. Mag. 180.

Results of the study and their discussion. In 92% of chronic gastritis were *Helicobacter pylori*-associated, among them one form or another prevailed.

Mainly superficial gastritis (fig. 1a), the initial atrophic gastritis (fig. 1b) were found in the body of the stomach.

In other parts of the stomach, pronounced atrophic gastritis (fig. 2a) prevailed, with varying degrees of severity in the pyloric region being $95.2 \pm 4.1\%$, in the minor curvature $88.3 \pm 6.2\%$ and in the body

58.8±8.5% of all observations. The expressed forms in the listed departments respectively amounted to 42.1±8.6%; 32.4±8.1%; 41.2±8.6%.

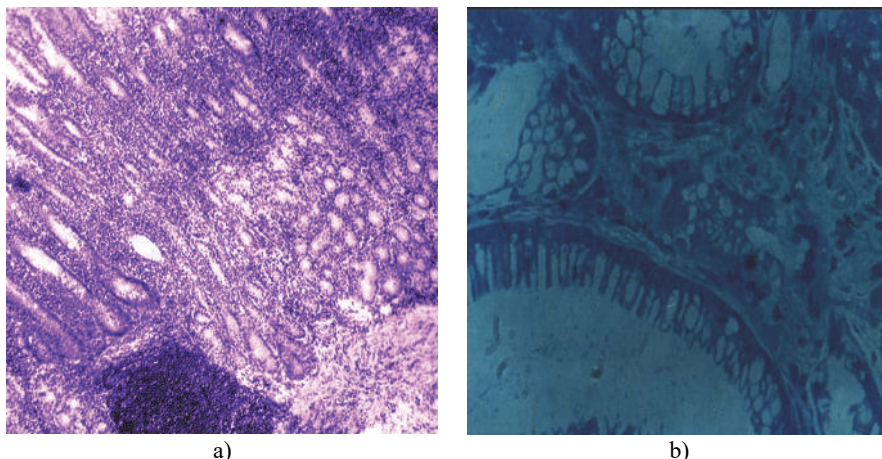


Fig.2: a) Pronounced atrophic gastritis. Atrophy of the glands. Lymphoid accumulations in the form of a follicle. Coloring hematoxylin-eosin. Mag. 180; b) Helicobacter pylori bacteria in the gastric mucosa. Semi-thin cut. Staining toluidine blue. Mag. 600.

The bacteria *Helicobacter pylori* were located within the pathogenic epithelium on the tops of the ridges, their lateral surface and deep in the pits. Often they were introduced between epithelial cells, violating the integrity of their layer (fig. 2b).

At these sites, the number of polymorphonuclear leukocytes of varying severity, based on the rollers in the general infiltrate that has lympho-

plasmacytic character, varied in the degree of severity.

Bacteria were found in all parts of the stomach, but a high degree of seeding was more often observed in the mucous membrane of the pyloric department and the lesser curvature of the stomach, compared with the mucous walls of the body of the stomach (42.2 ± 9.1 and 16.0 ± 6.0 ; $p < 0.05$).

Conversely, a low degree of infection of *Helicobacter pylori* was more often in the mucous membrane of the walls of the body of the stomach, compared with the mucous membrane of the pyloric department and the lesser curvature of the stomach (41.0 ± 10.1 and 15.0 ± 6.1 ; $p < 0.05$). A moderate degree of dissemination of *Helicobacter pylori* of the gastric mucosa in all its parts was evenly distributed.

The intensity of leukocyte infiltrate correlates with the degree of seeding of *Helicobacter pylori* of the gastric mucosa (fig. 3–4).

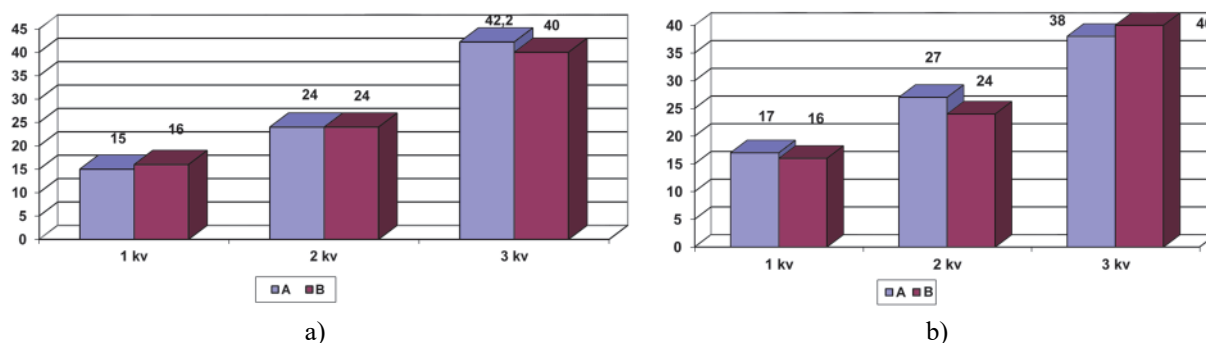


Fig. 3. a) Pyloric. A) The frequency of the various degrees of contamination of *Helicobacter pylori* of the gastric mucosa; B) Frequency of varying degrees of leukocyte infiltration: 1 kv–low, 2 kv–moderate, 3 kv–high degree in percent (%).

b) Small curvature. A) The frequency of the various degrees of contamination of *Helicobacter pylori* of the gastric mucosa; B) Frequency of varying degrees of leukocyte infiltration: 1 kv–low, 2 kv–moderate, 3 kv–high degree in percent (%).

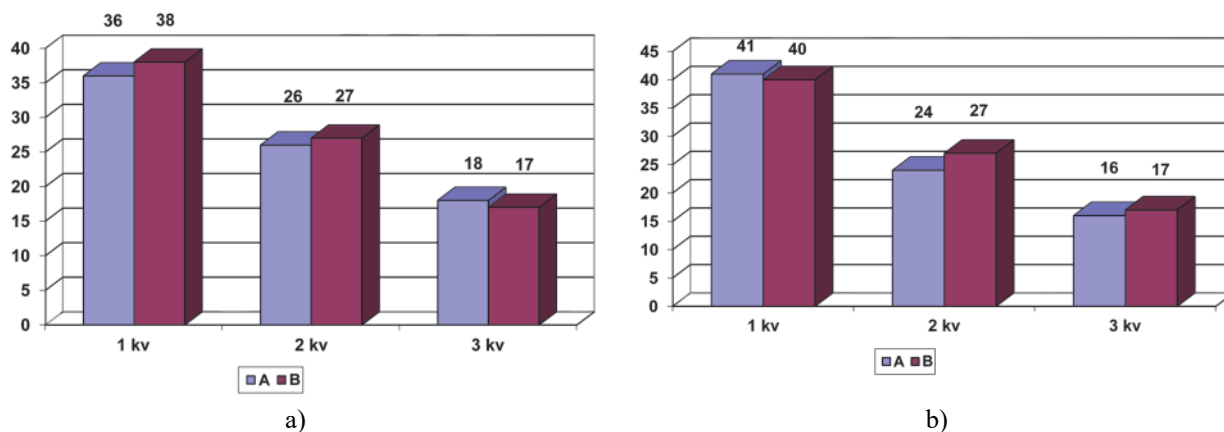


Fig. 4. a) The front wall of the body. A) The frequency of the various degrees of contamination of *Helicobacter pylori* of the gastric mucosa; B) Frequency of varying degrees of leukocyte infiltration: 1 kv–low, 2 kv–moderate, 3 kv–high degree in percent (%);

b) Rear wall of the body. A) The frequency of the various degrees of contamination of *Helicobacter pylori* of the gastric mucosa. B) Frequency of varying degrees of leukocyte infiltration: 1 kv–low, 2 kv–moderate, 3 kv–high degree in percent (%).

A decrease in the degree of bacterial contamination of the gastric mucosa from the pyloric region and the lesser curvature to the body walls was revealed. The degree of leukocyte infiltration decreases with a decrease in bacterial contamination.

Between the degree of contamination of the mucous membrane of *Helicobacter pylori* and the degree of leukocyte infiltration of the mucous membrane, the Pearson correlation coefficient r_{xy} is 0.935, the correlation is very strong, the determination coefficient is $D=r_{xy}^2=0.874$, the critical value of the correlation coefficient with a probability of 0.95–0.2732, the critical value of the correlation coefficient with a probability of 0.99–0.3511, comparing the correlation coefficient r_{xy} with a critical r_{cr} value for a significance of 0.95– $r_{xy} > r_{cr}$, comparing the correlation coefficient r_{xy} with a critical r_{cr} value for a significance of 0.99– $r_{xy} > r_{cr}$ to the covariance coefficient is 521.641, this makes it possible to conclude a statistically significant dependence with a probability of 0.99.

We consider it expedient, in discussing the results of our own research, to make a short theoretical discussion of this problem on the material of literary sources.

The central place among the pretumor diseases of the stomach is assigned to chronic gastritis, against the background of which and in connection with which precancerous changes occur [3]. One of the etiological factors of chronic gastritis, peptic ulcer and gastric cancer is *Helicobacter pylori*. According to the recommendations of the WHO special committee, we distinguish between precancerous conditions and precancerous changes. Precancerous conditions are diseases that increase the risk of cancer. But since 1994, *Helicobacter pylori* infection has been included in precancerous conditions [14].

Chronic gastritis is often found, especially among the population of young and working age, and represents an independent disease, or creates a background against which other diseases of the stomach develop, or accompanies them. A study by epidemiologists showed that gastric cancer in *Helicobacter pylori* infected people is more common than in uninfected [4].

Cellular update is regulated on the basis of negative feedback on the loss of part of the population. It is responsible for the increased neoplasm of cells and the acceleration of their migration. The reasons for reducing the population of epithelial cells can be their death on the spot and desquamation in the stomach cavity. As chronic gastritis progresses, more and more cells become in the S-phase, they even reach the tops of the rollers and can come into contact with carcinogens from the stomach cavity. *Helicobacter pylori* have the ability to stimulate cell renewal directly and by stimulating lymphocytes to synthesize relevant agents. In addition, *Helicobacter* gastritis usually leads to hypergastrinemia, which increases the proliferation of the epithelium of the stomach.

In countries with a high risk of gastric cancer, the impression of the *Helicobacter pylori* epithelium of the gastric mucosa occurs already in early childhood, and this suggests that a long-term infection may be the cause of the progression of chronic gastritis to gastric cancer [7].

Conclusion

In the gastric mucosa, pronounced forms of chronic atrophic gastritis associated with HP were found, respectively, 92% of cases. The degree of bacterial contamination correlates with the degree of leukocyte infiltration of the gastric mucosa. Chronic atrophic *Helicobacter pylori*-associated gastritis is a common disease of people at a young age involved in sports, and is central to pretumor diseases of the stomach.

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Реферати

РОЛЬ ХРОНІЧНОГО ГАСТРИТУ СЕРЕД ПЕРЕДРАКОВИХ ЗАХВОРЮВАНЬ ШЛУНКА

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Метою дослідження є статистичний аналіз стану слизової шлунка, враженого Helicobacter pylori, у молодих людей, які займаються спортом. Проведені дослідження хронічного гастриту типу В у студентів-добровольців, які займаються спортом. У 92% хронічні гастрити були Helicobacter pylori-асоційовані. Між ступенем обсіменіння слизової оболонки Helicobacter pylori і ступенем лейкоцитарної інфільтрації слизової оболонки коефіцієнт кореляції Пірсона $r_{xy} = 0,935$, тіснота зв'язку – дуже сильна, коефіцієнт детермінації $D = r_{xy}^2 = 0,874$, критичне значення коефіцієнта кореляції з вірогідністю 0,95–0,2732, критичне значення коефіцієнта кореляції з вірогідністю 0,99–0,3511, порівнюючи коефіцієнт кореляції r_{xy} з критичним значенням r_{cr} для значущості $0,95 - r_{xy} > r_{cr}$, порівнюючи коефіцієнт кореляції r_{xy} з критичним значенням r_{cr} для значущості $0,99 - r_{xy} > r_{cr}$. до коефіцієнта коваріації становить 521,641, це дозволяє зробити висновок про статистично значущу залежність з імовірністю 0,99.

Таким чином, хронічний атрофічний гастрит, асоційований з Helicobacter pylori, є поширеним захворюванням молодих людей, що займаються спортом, і є центральним серед передракових захворювань шлунку.

Ключові слова: Helicobacter pylori, гастрит, лейкоцитарна інфільтрація, слизова оболонка шлунка.
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РОЛЬ ХРОНИЧЕСКОГО ГАСТРИТА СРЕДИ ПРЕДРАКОВЫХ ЗАБОЛЕВАНИЙ ЖЕЛУДКА

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Целью работы является статистический анализ состояния слизистой оболочки желудка пораженной Helicobacter pylori у молодых людей, которые занимаются спортом. Проведены исследования хронического гастрита типа В у студентов-добровольцев, которые занимаются спортом. В 92% хронические гастриты были Helicobacter pylori-ассоциированные. Между степенью обсеменения слизистой оболочки Helicobacter pylori и степенью лейкоцитарной инфильтрации слизистой оболочки коэффициент корреляции Пирсона

$r_{xy} = 0,935$, плотность связи – очень сильная, коэффициент детерминации $D = r_{xy}^2 = 0,874$, критическое значение коэффициента корреляции с вероятностью 0,95–0,2732, критическое значение коэффициента корреляции с вероятностью 0,99–0,3511, сравнивая коэффициент корреляции r_{xy} с критическим значением r_{cr} для значимости $0,95 - r_{xy} > r_{cr}$, сравнивая коэффициент корреляции r_{xy} с критическим значением r_{cr} для значимости $0,99 - r_{xy} > r_{cr}$. к коэффициенту ковариации составляет 521,641, это позволяет сделать вывод о существовании статистически значимой зависимости с вероятностью 0,99.

Таким образом хронический атрофический Helicobacter pylori-ассоциированный гастрит является распространённым заболеванием людей в молодом возрасте, которые занимаются спортом, и занимает центральное место среди предопухолевых заболеваний желудка.

Ключевые слова: Helicobacter pylori, гастрит, лейкоцитарная инфильтрация, слизистая оболочка желудка.
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STUDY ON THE EFFECT OF THE VITAMIN AND MINERAL COMPLEX CONTAINING ZINC L-ASPARTATE ON THE PERIODONTAL CONDITION OF RATS IN THE PRESENCE OF PERIODONTITIS MODELING

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The purpose of the study was to study the effect of the vitamin and mineral complex containing zinc L-aspartate on the state of the periodontal tissues of rats under conditions of modeling periodontitis using exogenous collagenase. The vitamin and mineral complex containing zinc L-aspartate had a positive effect, to a greater extent, on the periodontal bone tissue. The complex has shown periodontal protection, anti-inflammatory, antioxidant properties.

Key words: zinc L-aspartate, periodontitis modeling, collagenase, collagen, glycosaminoglycans, gums, periodontal bone tissue, rats.

The study is a fragment of the research project "The effect of hypoxia on the processes of collagen formation and mineralization in models of dental pathology and correction of these disorders", state registration No. 0118U006963.

Extracellular matrix (ECM) of the connective tissue is defined by a complex system formed by multicellular structural macromolecules: proteoglycans, collagens, and elastins, which maintain its structural integrity. ECM consists of three essential components – a gelling medium, collagen and elastin fibers, and provides a rapid diffusion of substances and "construction" materials between blood and cells.

Changes in the state of the extracellular matrix (ECM) of periodontal tissues during periodontitis are carried out using matrix metalloproteinase (MMP_s) or collagenases, the main differences of which from other endopeptidases are associated with their ability to destroy the ECM structures of connective tissue, as well as with their dependence on metal ions. The balance between degradation and synthesis of ECM determines the state of periodontal soft tissues and bone tissues during periodontitis.

Zinc is essential in the body for cell growth, protein production, and wound healing. It helps to stabilize blood sugar levels, has a positive effect on the body's immune system, and exhibits antioxidant properties. Zinc is a part of more than 80 enzymes: carbonic anhydrase, RNA and DNA polymerases, carboxypeptidases.

Zinc is one of the most important trace elements that is part of many enzyme systems. It regulates the basic metabolic processes, participates in the metabolism of carbohydrates. Zinc is necessary for the functioning of more than 200 metalloenzymes (carbonic anhydrase, carboxypeptidase A, alkaline phosphatase, RNA polymerase, etc.), as well as for the normal structure of nucleic acids, proteins and cell membranes [2]. Zinc promotes cell growth and development, the normal functioning of the immune system and the provision of an immune response [11]. Zinc deficiency causes difficulties in concentration and memory, decreased cellular and humoral immunity, poor wound healing [4].

Zinc is a potent inhibitor of MMP_s and helps to improve the absorption of B vitamins. This element is important for the normal development of bone tissue. The accumulation of metals in connective tissue can affect the formation and absorption of extracellular components of ECM. It was found that Zn²⁺ ions have an inhibitory effect on MMP_s-2 and MMP_s-9. Studies of the effect of different ions (zinc, tin, copper, mercury) on gum gelatinase (MMP_s-2 and MMP_s-9) have shown that ZnSO₄ is its most potent inhibitor, while CuSO₄, HgSO₄, and others are less effective [10].

All of the above predetermined the study of the correction of damage to the extracellular matrix of the periodontium as a result of the periodontitis modeling with a complex of substances necessary for the normal functioning of the connective tissue.

The purpose of the study was to study the effect of the vitamin and mineral complex containing zinc L-aspartate on the state of the periodontal tissues of rats under conditions of modeling periodontitis using exogenous collagenase.

Materials and methods. The experiment was carried out on 21 white female breeding rats of the Wistar line. Animals of the 1st group (6 animal units) were intact. In Groups 2 and 3, periodontitis was modeled by injecting exogenous collagenase solution under the gums (from *Clostridium histolyticum* lyophilisat 2000 E/mg, Merk, Darmstadt (Germany) at a dose of 1 mg/ml in four areas of the jaws three times during the experiment. In the 3rd group, 7 rats were administered per os with the vitamin and mineral complex "Active Zinc" for 1 tablet/0.2 ml 5 times a week in the morning hours for 55 days, against the background of periodontitis modeling.

In addition to zinc L-aspartate, complex "Active Zinc" (TOV "Elit-PHARM", Dnipro, Ukraine) contains components that contribute to the better absorption of the zinc ion. Zinc is absorbed with the help of phosphorus, calcium, manganese, vitamins A, C, D₃.

1 tablet (0.250 g) of the complex contains zinc L-aspartate (active zinc – 12.6 mg), manganese aspartate (Mn²⁺ – 0.05 mg), calcium hydrogen phosphate (phosphorus – 30 mg), vitamin A – 1666 IU, vit. C – 10 mg, vit. D₃ – 2.5 mcg. Excipients: lactose, sorbitol, starch, calcium stearate.

At the end of the experiment, the rats were sacrificed by total exsanguination from the vessels of the heart under anesthesia with thiopental (40 mg/kg). All experiments were carried out in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental or other Scientific Purposes (Strasbourg, 1986). After separating the gums, the jaws were dissected out and the resorption of the alveolar bone was assessed morphometrically. The objects of biochemical studies were the gums and the alveolar bone of rats.

The ECM state of the connective tissue was determined by the levels of hydroxyproline (state of collagen) and glycosaminoglycans (GAGs). The lipid peroxidation level (LPL) was assessed by the content of malondialdehyde (MDA). The activity of antioxidant enzymes catalase [1], and glutathione peroxidase (GPx) was determined. To assess the state of rats' tissues, biochemical parameters were determined by unified methods using commercial reagent kits: alkaline phosphatase (ALP) activity, calcium (Ca²⁺), phosphorus, Mg²⁺, zinc, sialic acids. All kits were manufactured by DAC-SpectroMed, Moldova.

The experimental results were processed by conventional statistical methods with the determination of t-criteria for the reliability of differences according to Student's t-test.

Results of the study and their discussion. The study of the complex containing zinc L-aspartate effect on the periodontal tissues of rats was carried out under conditions of periodontitis modeling by subgingival administration of exogenous collagenase at a dose of 1 mg/ml. Oral administration of the

complex against the background of reproduced experimental periodontitis had a positive effect on the state of collagen in rats' periodontal tissues. Thus, under the influence of the complex, the free hydroxyproline level increased by 2.7 times ($p = 0.004$), the total level increased by 58% ($p = 0.003$) compared to the group "model of periodontitis", while the bound hydroxyproline level did not change significantly (table 1).

Table 1

Effect of the complex containing zinc L-aspartate on the state of the extracellular matrix (ECM) of rats' periodontium during the periodontitis modeling (M±m; p)

Studied indices	Groups of animals	
	periodontitis model (M)	M+"Active Zn"
	Gum	
Content: GAGs (mg/g)	3.87±0.59	3.55±0.32
hydroxyproline (µmol/g)		
free	2.12±0.41	5.65±0.87 $p=0.004$
bound	4.59±0.44	4.94±2.05
total	6.71±0.84	10.6±0.50 $p=0.003$
	alveolar bone	
Content: GAGs (mg/g)	2.12±0.074	2.56±0.055 $p=0.001$
hydroxyproline (µmol/g)		
free	1.41±0.21	2.06±0.00 $p=0.011$
bound	1.06±0.21	2.47±0.00 $p<0.001$
total	2.47±0.00	4.77±0.61 $p=0.005$

Note: In tables 1-3, the significance index p was calculated in comparison with the intact group.

Hydroxyproline content in the periodontal bone tissue also increased significantly: free – by 1.5 times ($p = 0.011$); bound – by 2.3 times ($p<0.001$); total – by 93% ($p = 0.005$). GAGs level in the alveolar bone increased by 21% ($p = 0.001$; table 1).

Under the influence of a complex containing zinc L-aspartate, the concentration of sialic acids in the blood serum decreased by 22% ($p = 0.004$; table 2), which indicates both its anti-inflammatory properties in the organism, and the partial restoration of glycoproteins, which indicates an improvement in the ECM state of the connective tissue.

Under the conditions of modeling periodontitis by subgingival administration of collagenases, the alveolar bone resorption in rats increased: in the mandible by 16% ($p < 0.001$) – 41.2±0.88% compared with the intact group: 35.5±0.90%. In the maxilla, the resorption increase was 20% ($p=0.016$): 31.3±1.43% versus 26.1±1.20% in the intact group (100%).

Table 2

Effect of a complex containing zinc L-aspartate on the content of sialic acids and metals in the periodontium connective tissue of rats in the modeling of periodontitis (M±m; p)

Studied indices	Groups of animals	
	periodontitis model (M)	M+"Active Zn"
	blood serum	
Content: sialic acids (mmol/ml)	2.40±0.059	1.88±0.096 $p=0.004$
	gum	
Content: Zn ²⁺ (µmol/g)	5.96±0.16	7.56±0.074 $p<0.001$
	alveolar bone	
Content: Mg ²⁺ (µmol/g)	0.098±0.0095	0.16±0.00 $p<0.001$
Zn ²⁺ (µmol/g)	2.09±0.28	2.44±0.39

Vitamin and mineral complex "Active Zinc" against the background of periodontitis model significantly reduced the resorption of periodontal bone tissue: by 14% in the mandible (100% in the control group; $p = 0.01$) and by 6% in the maxilla ($p > 0.05$; Table 3) compared with data from control groups. Thus, the periodontal protective properties of the complex containing zinc L-aspartate were revealed.

The content of Zn²⁺ increased in the soft tissues of the periodontium by 21% ($p < 0.001$); in the alveolar bone by 17% ($p > 0.05$; table 2). At the same time, the content of Mg²⁺ ions in the periodontal bone tissue significantly increased (by 63%; $p < 0.001$), which indicates an improvement in the state of the ECM in the alveolar bone. It is known that a lack of magnesium leads to a slowdown in protein synthesis. In addition, there was deterioration of the mechanical properties of the gel, which forms the ECM basic substance as a result of the hyaluronidase activation [3].

The results are quite justified in connection with the improvement of the mineral metabolism in this study object – an increase in alkaline phosphatase activity by 56% ($p = 0.02$) as a result of osteoblast activation, since alkaline phosphatase is their marker enzyme (table 3). The levels of calcium and phosphorus in these conditions increased insignificantly ($p > 0.05$; table 3).

Table 3

Effect of the complex containing zinc L-aspartate on the state of bone mineral metabolism in rats' periodontium during the periodontitis modeling (M±m; p)

Studied indices	Groups of animals	
	periodontitis model (M)	M+"Active Zn"
	alveolar bone	
Activity: ALP (nmol/s.g.)	0.27±0.035	0.42±0.035 p=0.002
Content: Ca ²⁺ (mmol/g)	0.024±0.0017	0.026±0.0046
Phosphorus (mmol/g)	0.023±0.0035	0.027±0.0052
Periodontal bone resorption indices (%)		
mandible	41.2±0.88	35.3±1.66 p=0.01
maxilla	31.3±1.43	29.5±1.31

Under the influence of the complex, the level of LPL processes decreased – the MDA content in the gums decreased by 14% (p = 0.02; table 4). At the same time, the activity of the antioxidant enzyme catalase in this study object increased by 28% (p = 0.05). Insufficient functioning of the antioxidant enzyme glutathione peroxidase in the gums was evidenced by a 1.9-fold decrease in its activity (p = 0.003) compared with the "Periodontitis model" group (table 4). In the periodontal bone tissue, the complex, more significantly than in the gums, reduced the level of peroxide products: the MDA content decreased by 2.2 times (p = 0.001), which may indicate the antioxidant properties of the complex containing zinc L-aspartate.

Catalase activity in the periodontal bone tissue under the complex influence changed insignificantly, while the activity of glutathione peroxidase increased by 6% (p = 0.06) compared with the control group (table 4).

Table 4

Effect of a complex containing zinc L-aspartate on the MDA content and the activity of antioxidant enzymes in the periodontium connective tissue of rats in the modeling of periodontitis (M±m; p)

Studied indices	Groups of animals	
	periodontitis model (M)	M+"Active Zn"
	gum	
Content: MDA (nmol/g)	50.3±1.02	43.1±2.51 p=0.02
Activity: catalase (mkat/g)	13.9±1.90	17.8±0.84 p=0.05
GPx (μmol/s.g.)	148±6.38	77.5±7.30 p=0.003
	alveolar bone	
Content: MDA (nmol/g)	0.80±0.083	0.37±0.012 p<0.001
Activity: catalase (mkat/g)	10.4±0.84	10.7±1.61
GPx (μmol/s.g.)	68.9±0.97	73.0±1.68 p=0.06

Studies have shown that the vitamin and mineral complex containing zinc L-aspartate, administered orally to rats against the background of reproduced experimental periodontitis, had a positive effect on the periodontal tissues of rats. First of all, under its influence, the state of collagen, recorded by the level of hydroxyproline (total, free, bound) was improved, which levels significantly increased compared to the control group (periodontitis model). In addition, in hard periodontal tissues, the complex restored the state of the gel, which forms the ECM basis of the connective tissue.

The zinc-containing complex significantly, by 1.6 times, increased the activity of alkaline phosphatase, an enzyme localized in bone tissue on the outer surface of osteoblast membranes. In this regard, the complex against the background of the periodontitis model significantly reduced the resorptive processes in the alveolar bone process of rats.

According to the literature, zinc is one of the components of enzymatic systems, which activity affects the growth, development and physiological state of the organism. It is referred to as trace elements that promote the synthesis of collagen, glycosaminoglycans. It is directly involved in the synthesis of bone matrix [13]. According to a number of scientists [9, 10, 11, 14], zinc increases bone formation, accelerates its mineralization, reduces bone resorption and stimulates the activity of alkaline phosphatase in osteoblast cell culture. Alkaline phosphatase is one of the most representative proteins of osteoblast differentiation as bone markers. As a result of these studies, the authors demonstrated the anabolic role of zinc in the formation of bone tissue under the action of osteoblasts [12, 14].

Zinc stimulates osteoblast proliferation and differentiation, as well as protein synthesis in osteoblasts. The importance of the zinc effect on the bone tissue metabolism is evidenced by information about its deficiency, in which the amount of bone mass decreases [8, 9]. Zinc deficiency contributes to the stress of DNA synthesis and protein metabolism, which leads to disruption of the organic matrix metabolism [1, 11].

Severe metabolic disorders of trace elements, including zinc, occur already in the early stages of the pathological process. Thus, the zinc content in the blood plasma of rats was reduced during the gingivitis and periodontitis reproduction, and more pronounced changes were observed in the group of rats with gingivitis [5].

The molecular mechanisms of zinc deficiency are based on oxidative stress in cells and tissues, as well as an increase in the synthesis of proinflammatory cytokines [7]. Zinc is involved in redox processes. Zinc cations stabilize the permeability of cell membranes and effect as a protector of free radical reactions.

The studied complex, containing zinc L-aspartate showed anti-inflammatory effect at the body level. It reduced the level of sialic acids in the blood serum, since it is known that an increase in the sialic acid content indicates an increase in inflammation in the tissues. It is known that sialic acids, derivatives of neuraminic acid, are formed under the action of neuraminidase during the breakdown of ECM glycoproteins. In our studies, the complex containing zinc L-aspartate showed antioxidant properties. It reduced the level of peroxide processes in the gums and alveolar bone. Under the complex influence, the Zn^{2+} content in the gums of experimental animals increased by 21%, in the bone tissue of the periodontium – by 17%.

According to the data, the optimal amount of zinc ions in the organism is an important factor that ensures the regenerative function of the connective tissue. During wound healing, zinc provides a stabilizing effect on the cytoplasmic membranes, preventing the hydrolytic enzyme release, such as cathepsin D and collagenase, which control the rate of damaged tissue degradation [6].

Thus, considering the positive results of experimental studies, the zinc content in the complex "Active Zinc" was optimal. The complex can be recommended as a means that promote bone tissue formation, has a direct effect on reducing alveolar bone resorption, and also replenishes the deficiency of the osteotropic trace element zinc, which is involved in vital metabolic processes of the organism.

Conclusions

1. Vitamin and mineral complex containing zinc L-aspartate, administered orally against the background of reproduced experimental periodontitis, increased by 58% the level of total hydroxyproline in the gums; in the alveolar bone – by 93%; the glycosaminoglycans content in the periodontal bone tissue increased by 21%.

2. The complex significantly (by 14%) reduced the alveolar bone resorption, which indicates its periodontal protective properties. It significantly improved mineral metabolism – increased the alkaline phosphatase activity in the periodontal bone by 56%.

3. Under the complex influence, the level of LPL processes in the gums and more significantly in the bone tissue of the periodontium decreased significantly; on the body level, the complex containing zinc L-aspartate reduced the concentration of sialic acids in the blood serum, which indicates its anti-inflammatory properties.

4. Vitamin and mineral complexes containing zinc L-aspartate can be recommended for improving the metabolism of periodontal connective tissue and bone mineral metabolism in periodontitis.

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Реферати

ВИВЧЕННЯ ВПЛИВУ ВІТАМІННО-МІНЕРАЛЬНОГО КОМПЛЕКСУ, ЩО МІСТИТЬ ЦИНК L-АСПАРАГІНАТ, НА СТАН ПАРОДОНТА ЩУРІВ В УМОВАХ МОДЕЛЮВАННЯ ПАРОДОНТИТУ

Шнайдер С.А., Балега М.І., Зомбор Є.В., Семенов Є.І., Ткаченко Є.К.

Метою дослідження було вивчення впливу вітамінно-мінерального комплексу, що містить цинк L-аспарагінат, на стан тканин пародонта щурів в умовах моделювання пародонтиту за допомогою екзогенної колагенази. Вітамінно-мінеральний комплекс, що містить цинк L-аспарагінат, мало позитивний вплив більшою мірою на кісткову тканину пародонту. Комплекс проявив пародонтопротекторні, протизапальні, антиоксидантні властивості.

Ключові слова: цинк L-аспарагінат, моделювання пародонтиту, колагеназа, колаген, глікозаміноглікани, ясна, кісткова тканина пародонту, щури.

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ИЗУЧЕНИЕ ВЛИЯНИЯ ВИТАМИННО-МИНЕРАЛЬНОГО КОМПЛЕКСА, СОДЕРЖАЩЕГО ЦИНК L-АСПАРАГИНАТ, НА СОСТОЯНИЕ ПАРОДОНТА КРЫС В УСЛОВИЯХ МОДЕЛИРОВАНИЯ ПАРОДОНТИТА

Шнайдер С.А., Балега М.И., Зомбор Е.В., Семенов Е.И., Ткаченко Е.К.

Целью исследования явилось изучение влияния витаминно-минерального комплекса, содержащего цинк L-аспарагінат, на состояние тканей пародонта крыс в условиях моделирования пародонтита с помощью экзогенной колагеназы. Витаминно-минеральный комплекс, содержащий цинк L-аспарагінат, оказал положительное влияние в большей степени на костную ткань пародонта. Комплекс проявил пародонтопротекторные, противовоспалительные, антиоксидантные свойства.

Ключевые слова: цинк L-аспарагінат, моделирование пародонтита, колагеназа, колаген, гликозаміноглікани, десна, костная ткань пародонта, крысы.

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EFFECT OF HORMONE-ACTIVE METABOLITES OF CHOLECALCIFEROL ON THE STATE OF THE ORAL CAVITY TISSUES IN RATS UNDER THE CONDITIONS OF ESTROGEN DEFICIENCY AND TRAUMATIC STRESS

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The purpose of the study was to comparatively establish the effect of active metabolites of vitamin D₃ on the periodontal condition of rats under conditions of experimental estrogen deficiency and traumatic stress. The experiment was carried out on 31 female breeding Wistar rats. 1st group was intact one (8 animals). The rats of the 2nd - 4th groups underwent ovariectomy and a femoral fracture. Group 2 was control one (8 rats); rats of the 3rd and 4th groups received per os preparations against the background of the pathogenic effect: Group 3 (8 rats) – 1- α -hydroxycholecalciferol at a dose of 0.1 μ g per day per rat; in the Group 4 (7 rats) – 24,25-hydroxycholecalciferol at a dose of 1.25 μ g per day/rat. At the time of sacrifice, the animals were 15 months old. Under the influence of risk factors for periodontitis, protective properties of hormone-active metabolites of vitamin D₃ were observed. They were expressed in inhibition of lipid peroxidation processes in the oral mucosa of rats, as well as periodontal protective effects when using 24,25-hydroxycholecalciferol.

Key words: vitamin D₃ metabolites, estrogen deficiency, traumatic stress, periodontal protection properties, antioxidant effect, rats.

The study is a fragment of the research project "The effect of hypoxia on the processes of collagen formation and mineralization in models of dental pathology and correction of these disorders", state registration No. 0118U006963.

Vitamin D₃ or cholecalciferol, which realizes its action in the organism through active metabolites – 25OHD₃, 1,25(OH)₂D₃ and 24,25(OH)₂D₃, is directly involved in the bone tissue metabolism. 1,25(OH)₂D₃ or calcitriol, is the most biologically active metabolite of vitamin D₃. With a deficiency of calcium and phosphorus, the metabolism of 25OHD₃ follows the formation of 1,25(OH)₂D₃, which is catalyzed by the enzyme 1- α -hydroxylase, which is present in the mitochondria of renal tubular epithelial cells. With an increased or normal concentration of calcium and phosphorus in the blood serum, an alternative metabolite, 25OHD₃ – 24,25(OH)₂D₃, is formed with 24-hydroxylase [10]. The fundamental

difference in the effects of $1.25(\text{OH})_2\text{D}_3$ from $24,25(\text{OH})_2\text{D}_3$ consists in the fact that in the first place is its resorption effect on the bone. At the same time, both metabolites have practically the same activity in relation to the absorption of calcium in the intestine. $24,25(\text{OH})_2\text{D}_3$ effects under physiological conditions, it ensures the calcium absorption in the intestine and utilization in the processes of mineralization and osteogenesis of bone tissue.

Recently, it has been shown that vitamin D_3 can affect the functioning of many organs and body systems. In addition to the well-known vitamin D_3 participation in the regulation of calcium and phosphorus metabolism and related effects in the processes of bone tissue remodeling, vitamin D_3 also has an immunomodulatory, anti-inflammatory, antiproliferative effect and is able to prevent tumor cell transformation [5]. Molecular mechanisms that provide the cytoprotective properties of vitamin D_3 can be realized both through genomic regulation, which mechanism mainly corresponds to the steroid hormone effects, and through non-genomic effects, including its influence on the expression of signaling proteins, cellular metabolism, inflammatory processes, and oxidative stress [11].

Vitamin D_3 is critical for the functioning of a wide variety of organ systems, and its deficiency contributes not only to low bone mineral density, osteoporosis, osteopenia, but also to infectious and chronic inflammatory diseases. It was found that the manifestations of systemic osteoporosis include an increase in atrophic processes in the jaw bones. A decrease in the level of $1.25(\text{OH})_2\text{D}_3$ in postmenopausal women plays an important role in the development of osteoporosis. The activity of $1\text{-}\alpha$ -hydroxylase is influenced by the level of estrogen.

In periodontitis, osteoclastic resorption of periodontal bone tissue increases, which is associated in the post-menopausal period in women with insufficient estrogen synthesis.

Among the synthetic analogs of $1.25(\text{OH})_2\text{D}_3$, $1\alpha\text{OND}_3$ ("Oxydevit", LLC "RPK EKHO", RF) is of the greatest importance. This form of vitamin D_3 has the same spectrum of action as $1.25(\text{OH})_2\text{D}_3$. Its high biological activity is explained by the conversion to $1.25(\text{OH})_2\text{D}_3$ as a result of hydroxylation involving 25OH . $1\alpha\text{OHD}_3$ has a more prolonged and "mild" effect.

The purpose of the study was to comparatively establish the effect of main active metabolites of cholecalciferol on the periodontal condition of rats under conditions of experimental estrogen deficiency and bone injury.

Materials and methods. The experiment was carried out on 31 female breeding Wistar rats. Group 1 (8 animals) consisted of intact rats. At 2 months of age, the rats of Groups 2-4 underwent ovariectomy, as well as a femoral fracture of one of the hind limbs 1 month before sacrifice. Group 2 (control) consisted of 8 rats (ovariectomy+fracture). Rats of the 3rd and 4th groups, starting from the next day at 2 months of age, were given per os preparations: the 3rd group (8 rats) – $1\alpha\text{OHD}_3$ at a dose of $0.1 \mu\text{g day/rat}$; in Group 4, 7 rats were injected with $24,25(\text{OH})_2\text{D}_3$ at a dose of $1.25 \mu\text{g day/rat}$ (LLC "RPK EKHO", RF). At the end of the experiment, the rats were sacrificed by total exsanguination from the vessels of the heart under anesthesia with thiopental (40 mg/kg). All experiments were carried out in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental or other Scientific Purposes (Strasbourg, 1986). Having previously separated the oral mucosa and submandibular salivary glands, maxilla and mandible were isolated. Resorption of the alveolar bone was assessed on freshly isolated jaws.

The objects of biochemical studies were the oral mucosa, submandibular salivary glands, alveolar bone, liver, femoral muscle, femur. The lipid peroxidation level (LPL) was assessed by the content of diene conjugates (DC) [4] and malondialdehyde (MDA). The activity of antioxidant enzymes was determined: glutathione reductase (GR) [8] and glutathione peroxidase (GPx) [12], as well as the state of the thiol-disulfide system [9]. In the liver, the content of DNA and RNA was determined [1]. The content of polyenoic fatty acids was determined in liver lipids by gas-liquid chromatography.

The experimental results were processed by conventional statistical methods with the determination of t-criteria for the reliability of differences according to Student's t-test.

Results of the study and their discussion. The study on the effect of the hormonal forms of cholecalciferol $1\alpha\text{OHD}_3$ and $24,25(\text{OH})_2\text{D}_3$ was carried out against the background of experimental ovariectomy in combination with traumatic stress of a femoral fracture (control group).

Morphometric studies of the alveolar process bone in rats showed that the experimental pathogenic effect did not significantly change the parameters of bone resorption of the periodontal bone (table 1).

Experimental ovariectomy performed in rats in combination with a femoral fracture significantly increased the level of diene conjugates in the liver by 5.4 times ($p < 0.001$) compared to the intact group, which indicates the intensification of LPL processes in this study object (Table 1). In the bone of the alveolar bone and in the femoral muscle, the increase in the level of the primary LPL products, diene conjugates, was not significant (3% and 52%, respectively, Table 2). In the control group, the activity of glutathione peroxidase in the liver increased by 1.5 times ($p = 0.05$) compared to the intact group, which

was apparently inductive in response to an increase in the amount of LPL products in this study object. In the femoral muscle, the activity of glutathione peroxidase was not significantly changed; glutathione reductase decreased by 25% ($p = 0.07$), which indicated insufficient functioning of glutathione metabolism enzymes in this study object (table 2).

LPL indices and the activity of glutathione metabolism enzymes were studied in the oral mucosa and submandibular salivary glands (Table 3). Thus, the MDA content in the control group is increased by 54% in comparison with the intact group ($p < 0.001$); in the submandibular salivary glands – by 81% ($p < 0.001$). The activity of glutathione peroxidase in the oral mucosa increased by 1.4 times ($p < 0.001$). Glutathione reductase activity did not change significantly (table 3). The study of nucleic acids content in the liver under conditions of ovariectomy and bone injury revealed the following: the level of DNA decreased by 5.6 times ($p < 0.001$); RNA – by 2.9 times ($p < 0.001$; Table 4). Experimental estrogen deficiency in combination with a traumatic fracture of the femur caused significant changes in the liver of rats in its fatty acid composition. Thus, the content of arachidonic acid (20:4) significantly decreased in liver lipids (by 66%; $p = 0.001$): $7.81 \pm 0.62\%$ versus $11.7 \pm 1.0\%$ in the intact group. The level of eicosapentaenoic acid (20:5) in the control group decreased by 65% (trend; $p = 0.10$): $1.21 \pm 0.18\%$ versus $1.86 \pm 0.33\%$. The content of docosahexaenoic acid (22:6) significantly decreased by 67% ($p = 0.011$): $4.61 \pm 0.25\%$ versus $6.90 \pm 0.65\%$ in the intact group.

Morphometric study of periodontal bone resorption showed that $1\alpha\text{OHD}_3$ did not significantly affect, and $24,25(\text{OH})_2\text{D}_3$ reduced the alveolar bone resorption in rats by 12% (trend; $p_1 = 0.08$; 100% in the control group; table 1).

Table 1

The effect of vitamin D₃ metabolites on the resorption parameters (%) of the alveolar bone in rats (M±m; p₁)

Studied index	Groups of animals			
	1	2	3	4
Alveolar bone resorption (%) (mean values)	43.0±1.5	43.5±1.6	42.4±1.1	38.2±2.3 $p_1=0.08$

Notes. In table 1, the reliability index p is calculated in comparison with the control group ("ovariectomy+fracture")

Of the two studied hormonal forms of vitamin D₃, $24,25(\text{OH})_2\text{D}_3$ significantly reduced ($p < 0.001$) the level of the primary LPL products – diene conjugates in the liver of rats and doubled ($p_1 < 0.001$) the activity of glutathione peroxidase, which indicates an overall positive effects on the organism of rats (table 2). The content of diene conjugates in the femoral muscle decreased to a greater extent $1\alpha\text{OHD}_3$ than $24,25(\text{OH})_2\text{D}_3$ (Table 2). $1\alpha\text{OHD}_3$ increased the activity of glutathione peroxidase by 55% ($p_1 < 0.001$). $24,25(\text{OH})_2\text{D}_3$ increased the activity of glutathione reductase by 17% ($p_1 < 0.002$; table 2).

Table 2

The effect of vitamin D₃ metabolites on the LPL state and the activity of antioxidant enzymes in rats' tissues (M±m; p; p₁)

Studied index	Groups of animals			
	1	2	3	4
	liver			
Content: DC (extinction units/g)	0.37±0.13	2.00±0.010 $p < 0.001$	1.86±0.11	0.16±0.090 $p_1 < 0.001$
Activity: GR (nmol/s.g.)	0.42±0.13	0.47±0.099	1.43±0.90	–
GPx (nmol/s.g.)	8.10±0.92	12.5±2.20	14.0±3.86	26.6±0.64 $p_1 < 0.001$
	femoral muscle			
Content: DC (extinction units/g)	0.33±0.11	0.50±0.14	0.054±0.015 $p_1 = 0.006$	0.17±0.049 $p_1 = 0.04$
Activity: GR (nmol/s.g.)	3.90±0.48	2.91±0.12 $p = 0.07$	1.25±0.23 $p_1 < 0.001$	3.41±0.013 $p_1 = 0.002$
GPx (nmol/s.g.)	12.6±0.83	13.3±0.46	20.6±1.012 $p_1 < 0.001$	13.2±5.61
	alveolar bone			
Kinetics of MDA accumulation (%) 1st hour of incubation	114±2.7	117±4.7	124±3.8	134±99.2
2nd hour of incubation	121±2.4	129±4.2	162±55.1	145±9.3
	femoral bone			
Kinetics of MDA accumulation (%) 1st hour of incubation	–	127±5.9	178±7.5 $p_1 < 0.001$	100±0.00 $p_1 = 0.001$
2nd hour of incubation	–	147±8.2	165±0.00 $p_1 = 0.05$	175±23.4

Notes. In tables 2-5, the reliability index p was calculated in comparison with the intact group; p_1 – compared to the control ("ovariectomy+fracture")

The parameters of the kinetics of MDA accumulation under the influence of the studied drugs in the femur underwent significant changes (table 2). So, if $1\alpha\text{OHD}_3$ increased the kinetics of MDA accumulation during 1- and 2-hours incubation, then 24,25-dioxycholecalciferol decreased this index by 21% even after 1-hour incubation ($p_1 = 0.001$). Hormone-active forms of cholecalciferol under these experimental conditions did not significantly change the kinetics of MDA accumulation in the alveolar bone (table 2).

Table 3

The effect of vitamin D₃ metabolites on the LPL state and the activity of antioxidant enzymes in rats' oral tissues (M±m; p; p₁)

Studied indices	Groups of animals			
	1	2	3	4
	oral mucosa			
Content: MDA (μmol/g)	0.041±0.0010	0.063±0.0056 p=0.001	0.035±0.0075 p ₁ = 0.011	0.033±0.0075 p ₁ = 0.008
Activity: GR (nmol/s.g.)	3.40±0.098	3.37±0.096	1.76±0.037 p ₁ < 0.001	3.03±0.25
GPx (nmol/s.g.)	12.9±0.38	17.5±2.06 p=0.04	23.6±1.32 p ₁ = 0.02	-
	submandibular salivary glands			
Content: MDA (μmol/g)	0.054±0.005	0.098±0.0038 p < 0.001	0.096±0.0052	0.10±0.018
Activity: GR (nmol/s.g.)	0.61±0.097	0.67±0.054	0.21±0.046 p ₁ < 0.001	1.01±0.085 p ₁ = 0.005
GPx (nmol/s.g.)	16.4±0.89	23.2±1.03 p < 0.001	27.8±0.79 p ₁ = 0.004	31.1±1.01 p ₁ < 0.001

We studied the changes in LPL indices and the activity of glutathione metabolism enzymes were studied in the oral mucosa and submandibular salivary glands (table 3). The content of MDA under the influence of $1\alpha\text{OHD}_3$ and 24.25 (OH)₂D₃ decreased in the oral mucosa by 1.8 and 1.9 times ($p_1 = 0.008$), respectively. Both metabolites did not significantly change the studied parameter in the submandibular salivary glands in comparison with the data of the control groups (table 3). $1\alpha\text{OHD}_3$ increased by 35% ($p_1 = 0.02$) glutathione peroxidase activity in the oral mucosa and by 14% ($p_1 = 0.004$) in the submandibular salivary glands and significantly reduced the activity of glutathione reductase in submandibular salivary glands as compared to the control groups. 24.25(OH)₂D₃ significantly increased the activity of both glutathione metabolism enzymes in the submandibular salivary glands (table 3).

Under the influence of $1\alpha\text{OHD}_3$, the DNA content in the liver increased by 2.9 times ($p_1=0.02$; table 4). The RNA level increased by 3.4 times ($p_1<0.001$) under the influence of $1\alpha\text{OHD}_3$ and 24.25(OH)₂D₃ compared to the control groups (table 4).

Table 4

The effect of vitamin D₃ metabolites on the content of RNA and DNA in the liver of rats (M±m; p; p₁)

Studied indices	Groups of animals			
	1	2	3	4
Content (mcg/g): DNA	151±9.90	27.1±16.0 p<0.001	79.8±11.5 p ₁ = 0.02	54.2±11.5
RNA	151±4.30	52.1±8.70 p<0.001	179±19.5 p ₁ <0.001	175±20.1 p ₁ <0.001

The study of the fatty acid composition of liver lipids under the influence of preparations of hormonal forms of cholecalciferol revealed the following. Thus, $1\alpha\text{OHD}_3$ increased the arachidonic acid content(20:4) by 36% ($p_1=0.08$):10.6±1.3% vs. 7.81±0.62%; 24,25 (OH)₂D₃ – by 33% ($p_1 = 0.05$): 10.4±1.0% vs. 7.81±0.62% compared to the control groups. This active metabolite significantly increased the level of oleic acid (18:2) by 19% ($p_1 = 0.04$): 27.0±1.6% versus 22.7±0.9% and docosahexaenoic acid (22:6) by 25%: 5.75±0.53% versus 4.61±0.25% (trend; $p_1 = 0.08$).

Under the influence of risk factors for the periodontitis development (aging and chronic traumatic stress), the protective properties of hormonally active metabolites of vitamin D₃ were observed. They were expressed in the inhibition of LPL processes in the oral mucosa of rats, as well as periodontal protective effects when testing 24.25-dihydroxy vitamin D₃.

In the course of the studies, it was found that under the influence of risk factors for periodontitis (aging and chronic traumatic stress), LPL processes were activated both at the level of the body – in the liver, and in the tissues of the oral cavity – in the oral mucosa and submandibular salivary glands. The high biological activity of peroxidation products in biomolecules necessitates the constant functioning of a

special mechanism of antioxidant system in the cells, the most important components of which are antioxidant enzymes [3, 14].

The increase in glutathione peroxidase activity in the liver, oral mucosa and submandibular salivary glands was compensatory in response to the activation of peroxide processes in these study objects. An excess of peroxides can contribute to the oxidative destruction of not only lipids, but also proteins. It is generally accepted that the oxidative modification of proteins plays a key role in the molecular mechanisms of oxidative stress and may be a trigger for damage to other biomolecules (DNA and RNA) of the cell. Thus, in our studies, ovariectomy with bone injury caused a significant decrease in the levels of RNA and DNA in liver cells, as well as changes in the fatty acid composition of its lipids, apparently as a result of peroxide destruction.

In our experiments, hormonal forms of vitamin D₃ – 1 α OHD₃ and 24.25 (OH)₂D₃ were used in conditions of ovariectomy and chronic bone injury. The protective properties of the two hormonal forms were expressed in the inhibition of LPL processes in the oral mucosa, as well as in the activity of glutathione metabolism enzymes – glutathione reductase and glutathione peroxidase in the submandibular salivary glands. Both hormonal forms of vitamin D₃ increased the level of RNA, and 1 α OHD₃ increased the DNA content in the liver. 24.25(OH)₂D₃ significantly reduced the level of LPL processes in the liver and activated glutathione peroxidase, which provides protection against the damaging effects of peroxides of different nature. A decrease in resorptive processes in the periodontal bone tissue caused 24.25 (OH)₂D₃; in the femur – a significant decrease in LPL processes.

Insufficient anti-resorptive effect of 1 α -dihydroxyvitamin D₃, as well as an increase in MDA level in the femur (in case of its fracture), on the one hand, are associated with a significant pathogenic effect, as well as the fact that metabolizing, 1 α OHD₃ is converted into calcitriol gradually. At the same time, the biological activity of 1 α -dihydroxyvitamin D₃ in rats and humans is only half the activity of the hormonal form of 1,25-dioxy-vitamin D₃. In the liver, some leveling of the consequences of the peroxidation syndrome was observed, in particular, a partial restoration of the fatty acid composition of liver lipids when using 1 α OHD₃ and, to a greater extent, under the influence of 24,25 (OH)₂D₃. Thus, the most pronounced antioxidant properties in selected experimental conditions at the level of the rats' organism and in the tissues of the oral cavity were shown by 24.25-dioxy-vitamin D₃.

The data obtained confirm the presence of antioxidant activity inherent in the vitamin D₃ molecule itself [2]. It is assumed that cholecalciferol can act as a membrane antioxidant, stabilizing membranes and protecting them from LPL through interaction with their hydrophobic regions [6, 11]. According to the literature, calcitriol (1.25 (OH)₂D₃) can enhance the elimination of reactive oxygen and nitrogen forms, increasing the intracellular pool of reduced glutathione. In addition, the effects of vitamin D₃ may be mediated by its anti-inflammatory activity through gene transcription of numerous anti-inflammatory cytokines [12, 13].

Conclusions

1. Under conditions of ovariectomy reproduction and bone trauma LPL processes in the liver, oral mucosa and submandibular salivary glands were intensified; the levels of DNA and RNA in the liver decreased, as well as the content of arachidonic acid as a result of its peroxide destruction.
2. Hormone-active metabolites of vitamin D₃ under the influence of risk factors for periodontitis showed an antioxidant effect, and to a greater extent the metabolite 24,25-dioxycholecalciferol.
3. 24,25(OH)₂D₃ inhibited LPL processes in the liver, femur and oral mucosa; in the submandibular salivary glands it increased the activity of glutathione peroxidase; in the liver it restored RNA, as well as the fatty acid composition of its lipids. 24,25(OH)₂D₃ showed a periodontal protective effect.

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Реферати

ВПЛИВ ГОРМОНАЛЬНО-АКТИВНИХ МЕТАБОЛІТІВ ХОЛЕКАЛЬЦИФЕРОЛУ НА СТАН ТКАНИН РОТОВОЇ ПОРОЖНИНИ ЩУРІВ В УМОВАХ ЕСТРОГЕННОЇ НЕДОСТАТНОСТІ І ТРАВМАТИЧНОГО СТРЕСУ

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Метою дослідження було порівняльне вивчення впливу активних метаболітів вітаміну D₃ на стан пародонту щурів в умовах експериментальної естрогенної недостатності та травматичного стресу. Дослідження проведено на 31 щурі-самці лінії Вістар стадного розведення. 1-я група – інтактна (8 особин). Щурам 2-ї – 4-ї груп була проведена оваріектомія і перелом стегнової кістки. 2-я група – контрольна (8 щурів); щури 3-ї – 4-ї груп на тлі проведеного патогенного впливу отримували препарати per os: 3-ї групи (8 щурів) – 1 α ОНD₃ в дозі 0,1 мкг в день на щура; в 4-ї групі (7 щурів) – 24,25 (ОН)₂D₃ в дозі 1,25 мкг в день/щура. На момент виведення з експерименту тварини знаходилися у віці 15 місяців. В умовах впливу факторів ризику пародонтиту спостерігалися захисні властивості гормонально-активних метаболітів вітаміну D₃, що виразилися в гальмуванні процесів перекисного окислення ліпідів у слизовій оболонці порожнини рота щурів, а також пародонтопротекторна ефекти при застосуванні 24,25-діоксिवітаміна D₃.

Ключові слова: метаболіти вітаміну D₃, естрогенна недостатність, травматичний стрес, пародонтопротекторні властивості, антиоксидантна дія, щури.

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ВЛИЯНИЕ ГОРМОНАЛЬНО-АКТИВНЫХ МЕТАБОЛИТОВ ХОЛЕКАЛЬЦИФЕРОЛА НА СОСТОЯНИЕ ТКАНЕЙ РОТОВОЙ ПОЛОСТИ КРЫС В УСЛОВИЯХ ЭСТРОГЕННОЙ НЕДОСТАТОЧНОСТИ И ТРАВМАТИЧЕСКОГО СТРЕССА

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Целью исследования явилось сравнительное изучение влияния активных метаболитов витамина D₃ на состояние пародонта крыс в условиях экспериментальной эстрогенной недостаточности и травматического стресса. Опыт проведен на 31 крысе-самке линии Вистар стадного разведения. 1-я группа – интактная (8 особей). Крысам 2-й – 4-ой групп были проведены оваризэктомия и перелом бедренной кости. 2-я группа – контрольная (8 крыс); крысы 3-й – 4-ой групп, на фоне проведенного патогенного воздействия, получали препараты per os: 3-й группы (8 крыс) – 1 α ОНD₃ в дозе 0,1 мкг в день на крысу; в 4-й группе (7 крыс) – 24,25(ОН)₂D₃ в дозе 1,25 мкг в день/крысу. На момент выведения из эксперимента животные находились в возрасте 15 месяцев. В условиях воздействия факторов риска пародонтита наблюдались защитные свойства гормонально-активных метаболитов витамина D₃, выразившиеся в торможении процессов перекисного окисления липидов в слизистой оболочке полости рта крыс, а также пародонтопротекторные эффекты при применении 24,25-диоксивитамина D₃.

Ключевые слова: метаболиты витамина D₃, эстрогенная недостаточность, травматический стресс, пародонтопротекторные свойства, антиоксидантное действие, крысы.

Рецензент Єрошенко Г.А.

ШАНОВНІ КОЛЕГИ!

Матеріали для опублікування приймаються від спеціалістів у галузі теоретичної, профілактичної, клінічної медицини, суміжних дисциплін, а також досліджень в галузі біологічних наук (Наказ МОН України № 612 від 07.05.2019 р.). Мова публікацій – українська, англійська, російська.

Публікація повинна відбивати сучасний стан розробки досліджуваної проблеми, містити нові результати на основі проведеного дослідження, **перспективи подальших розробок у даному напрямку**. Висновки мають бути аргументованими відповідним ілюстративним матеріалом.

До друку приймаються наукові статті, які містять такі необхідні елементи: шифр УДК; назва статті; ініціали авторів та прізвища (кількість авторів однієї статті не повинна перевищувати п'яти осіб); назва установи та місто; дані про **зв'язок публікації з плановими науково-дослідними роботами** (з наведенням номеру держреєстрації)

Вступ: постановка проблеми у загальному вигляді; аналіз останніх (за останні 10 років) досліджень та публікацій, в яких започатковано розв'язання даної проблеми і на які спирається автор; визначення невирішених раніше частин загальної проблеми;

Мету

Матеріал та методи дослідження

Результати дослідження та їх обговорення

Висновки або підсумки

Перспективи подальших розробок у даному напрямку

Список літератури за Ванкуверським стилем спочатку кирилицею, потім – латиницею.

Реферати російською, українською і англійською мовами обсягом не більше 0,5 стор. У випадку наведення статті українською, або російською мовами додаткове резюме англійською мовою – 1,5-2 сторінки англійською мовою, англійською мовою – розширене резюме українською мовою.

Таблиці – не більше 4. Графіки (**не більше 4**) повинні мати чіткі калібрування по осям. Якщо наводяться декілька кривих, безпосередньо на рисунку необхідно вказати їх порядкові номери. Рисунки, крім діаграм, в електронному варіанті бажано надавати в розширенні РСХ, фото (**не більше 4**) – в JPEG.

Список літератури оформлюється без скорочень. Автори подаються за абеткою, спочатку кирилицею, потім латиницею. Посилання в тексті зазначаються цифрами у квадратних дужках.

Статті редагуються і рецензуються членами редакційної ради – провідними фахівцями з відповідних галузей біології та медицини.

Текст набирається через 1,5 інтервал **без переносів**, розмір шрифту 14 у Times New Roman в редакторі MICROSOFT WORD. **Відступ абзацу – 1,25 см знаком табуляції**. Поля з усіх боків по 25 мм. Матеріал потрібно надавати на електронну пошту – womab.ed@gmail.com.

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