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ЕЖЕМЕСЯЧНЫЙ НАУЧНЫЙ ЖУРНАЛ

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ЕЖЕМЕСЯЧНЫЙ НАУЧНЫЙ ЖУРНАЛ
ТБИЛИСИ - НЬЮ-ЙОРК

GMN: Georgian Medical News is peer-reviewed, published monthly journal committed to promoting the science and art of medicine and the betterment of public health, published by the GMN Editorial Board and The International Academy of Sciences, Education, Industry and Arts (U.S.A.) since 1994. **GMN** carries original scientific articles on medicine, biology and pharmacy, which are of experimental, theoretical and practical character; publishes original research, reviews, commentaries, editorials, essays, medical news, and correspondence in English and Russian.

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3. Submitted material must include a coverage of a topical subject, research methods, results, and review.

Authors of the scientific-research works must indicate the number of experimental biological species drawn in, list the employed methods of anesthetization and soporific means used during acute tests.

4. Articles must have a short (half page) abstract in English, Russian and Georgian (including the following sections: aim of study, material and methods, results and conclusions) and a list of key words.

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3. სტატიაში საჭიროა გაშუქდეს: საკითხის აქტუალობა; კვლევის მიზანი; საკვლევი მასალა და გამოყენებული მეთოდები; მიღებული შედეგები და მათი განსჯა. ექსპერიმენტული ხასიათის სტატიების წარმოდგენისას ავტორებმა უნდა მიუთითონ საექსპერიმენტო ცხოველების სახეობა და რაოდენობა; გაუტკივარებისა და დაძინების მეთოდები (მწვავე ცდების პირობებში).

4. სტატიას თან უნდა ახლდეს რეზიუმე ინგლისურ, რუსულ და ქართულ ენებზე არანაკლებ ნახევარი გვერდის მოცულობისა (სათაურის, ავტორების, დაწესებულების მითითებით და უნდა შეიცავდეს შემდეგ განყოფილებებს: მიზანი, მასალა და მეთოდები, შედეგები და დასკვნები; ტექსტუალური ნაწილი არ უნდა იყოს 15 სტრიქონზე ნაკლები) და საკვანძო სიტყვების ჩამონათვალი (key words).

5. ცხრილები საჭიროა წარმოადგინოთ ნაბეჭდი სახით. ყველა ციფრული, შემაჯამებელი და პროცენტული მონაცემები უნდა შეესაბამებოდეს ტექსტში მოყვანილს.

6. ფოტოსურათები უნდა იყოს კონტრასტული; სურათები, ნახაზები, დიაგრამები - დასათაურებული, დანომრილი და სათანადო ადგილას ჩასმული. რენტგენოგრაფიების ფოტოასლები წარმოადგინეთ პოზიტიური გამოსახულებით **tiff** ფორმატში. მიკროფოტოსურათების წარწერებში საჭიროა მიუთითოთ ოკულარის ან ობიექტივის საშუალებით გადიდების ხარისხი, ანათალების შედეგების ან იმპრეგნაციის მეთოდი და აღნიშნოთ სურათის ზედა და ქვედა ნაწილები.

7. სამამულო ავტორების გვარები სტატიაში აღინიშნება ინიციალების თანდართვით, უცხოურისა – უცხოური ტრანსკრიპციით.

8. სტატიას თან უნდა ახლდეს ავტორის მიერ გამოყენებული სამამულო და უცხოური შრომების ბიბლიოგრაფიული სია (ბოლო 5-8 წლის სიღრმით). ანბანური წყობით წარმოდგენილ ბიბლიოგრაფიულ სიაში მიუთითეთ ჯერ სამამულო, შემდეგ უცხოელი ავტორები (გვარი, ინიციალები, სტატიის სათაური, ჟურნალის დასახელება, გამოცემის ადგილი, წელი, ჟურნალის №, პირველი და ბოლო გვერდები). მონოგრაფიის შემთხვევაში მიუთითეთ გამოცემის წელი, ადგილი და გვერდების საერთო რაოდენობა. ტექსტში კვადრატულ ფხიხლებში უნდა მიუთითოთ ავტორის შესაბამისი N ლიტერატურის სიის მიხედვით. მიზანშეწონილია, რომ ციტირებული წყაროების უმეტესი ნაწილი იყოს 5-6 წლის სიღრმის.

9. სტატიას თან უნდა ახლდეს: ა) დაწესებულების ან სამეცნიერო ხელმძღვანელის წარდგინება, დამოწმებული ხელმოწერითა და ბეჭდით; ბ) დარგის სპეციალისტის დამოწმებული რეცენზია, რომელშიც მითითებული იქნება საკითხის აქტუალობა, მასალის საკმაობა, მეთოდის სანდოობა, შედეგების სამეცნიერო-პრაქტიკული მნიშვნელობა.

10. სტატიის ბოლოს საჭიროა ყველა ავტორის ხელმოწერა, რომელთა რაოდენობა არ უნდა აღემატებოდეს 5-ს.

11. რედაქცია იტოვებს უფლებას შეასწოროს სტატია. ტექსტზე მუშაობა და შეჯერება ხდება საავტორო ორიგინალის მიხედვით.

12. დაუშვებელია რედაქციაში ისეთი სტატიის წარდგენა, რომელიც დასაბეჭდად წარდგენილი იყო სხვა რედაქციაში ან გამოქვეყნებული იყო სხვა გამოცემებში.

აღნიშნული წესების დარღვევის შემთხვევაში სტატიები არ განიხილება.

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HAYKA

SURGICAL TREATMENT OF COMBINED OCCLUSIVE-STENOTIC LESIONS OF EXTRACRANIAL ARTERIES AND AORTO/ILIAC-FEMORAL SEGMENT IN CONDITIONS OF HIGH RISK OF DEVELOPMENT OF REPERFUSION-REOXYGENATIVE COMPLICATIONS

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The atherosclerotic process simultaneously affects several vascular basins and forms a characteristic clinical picture of ischemic manifestations of various organs and systems - from explicit manifestation to asymptomatic, latent leakage in others [1]. Pathogenetic validity and clinical efficacy of surgical treatment of hemodynamically significant lesions of the major arteries of the lower extremities, brachiocephalic and coronary arteries have been proved in a number of studies [2]. The decision of the order of restorative operations on the brachiocephalic arteries and the aorto/iliac-femoral segment is based on the analysis of the localization of the occlusive process in both arterial basins, their hemodynamic characteristics, the degree of ischemia and the tolerance of the brain to hypoxia [3]. Depending on the results of the analysis, two surgical tactics, one-stage or stage-by-stage, are adhered to during revascularization operations in both arterial basins [4]. In patients with atherosclerotic occlusion of the aorta/iliac-femoral arterial bed in conditions of chronic arterial insufficiency of the lower extremities of IIIA-B - IVst. (according to the classification of Fontaine R. taking into account the criteria of the European Working Group (1992) there is a high risk of developing of reperfusion-reoxygenative complications [6]. According to the mentioned, the revascularization of the extracranial arterial basin in patients with combined lesions of both arterial regions with a high risk of reperfusion-reoxygenative syndrome should be performed before the reconstruction of the aorta/iliac-femoral basin or simultaneously.

The aim of the study - improve the results of surgical treatment of combined occlusion-stenotic lesions of extracranial and aortic/iliac-femoral arterial pools under conditions of high risk of reperfusion-reoxygenative syndrome.

Material and methods. 58 patients with combined lesions of extracranial and aortic/iliac-femoral arterial basins were examined and operated.

Age of patients in the range of 52 - 74 years (average age - 63,4 + 7,4 years), all male patients.

In all patients were diagnosed different variants of atherosclerotic occlusion of the aorta/iliac-femoral segment. Of these, 31 patients had a stenotic-occlusive process of the femoral-distal arterial lumen of the lower extremities, and 6 were the stenotic-occlusive process of the femoral-poplite segment of only one lower limb. One of the lower extremities was characterized by IIIA-B st. of chronic arterial deficiency (CAD), the contralateral lower limb - IIA-B st. of CAD (according to the classification of Fontaine R. taking into account the criteria of the European Working Group (1992).

Among 58 patients in four was noticed the occlusive-stenotic atherosclerotic processes of the brachiocephalic trunk (BCT) and in three we noticed the stenosis (69-76%) of bifurcation of the common carotid artery (CCA) with its expansion on the internal carotid artery (ICA) were revealed. Atherosclerotic lesions of the ICA were diagnosed in 51 patients (ICA stenosis at the level of 64 - 90%). In 17 of them, it was combined with the stenotic process (26 - 37%).

In 24 (41.4%) patients, the compensated stage of cerebrovascular failure was diagnosed, and 34 (58.6%) were at the stage of relative compensation. The compensated stage of cerebrovascular failure was characterized by an asymptomatic course or its initial manifestations. The stage of relative compensation was manifested by transient violations of cerebral blood circulation - in 15 (44.1%) of observations, dyscirculatory encephalopathy - in 14 (41.7%) patients, and transient ischemic attacks (up to two episodes) were in 5 (14.7%) patients.

Ultrasound system "Ultramark-9" (USA) and "Doptek" (England) were used for diagnostics of hemodynamics of extracranial arteries, for the diagnosis of hemodynamics of the aorta and trunk arteries of the lower extremities - ultrasound system Siemens Acuson S2000 (Germany). X-ray contrast digital angiography was used to determine the structural and hemodynamic characteristics of the aorta and trunk arteries of the lower extremities.

The system of preoperative preparation introduced a set of preventive measures reperfusion-reoxygenative syndrome, which included: the elimination of vasoconstriction by conducting prolonged epidural anesthesia and infusion of prostaglandins (alprostadil); reduction of neutrophilic activity and level of systemic inflammatory response by conducting a session of leukapheresis; increasing of the endothelium resistance by the prescribing of a beta-blocker (nebivolol), perindopril and L-arginine; for the normalization of microvascular permeability we used salt hyperosmotic hypertonic solution (dextran); the increase of the activity of prooxidant-antioxidant equilibrium was achieved by the introduction of alpha-tocopherol and corvutin; in order to achieve anti-ischemic effect, intravenous infusion of hydroxyethyl starch was prescribed.

Results and their discussion. 51 patients underwent the one-time surgical intervention on extracranial arteries and aorto/iliac-femoral segments. In all observations, the surgical intervention on extracranial vessels was carried out in the form of carotid endarterectomy, and in the aorto/iliac-femoral zone - in the form of aorto-bifemoral alos hunting.

In the spread occlusion-stenotic lesion of BCT and bifurcation of CCA and ICA in combination with the stenotic process of the contralateral ICA in the stage of relative compensation of cerebral blood flow in 7 patients, primarily surgical intervention on the vessels of the neck was performed. Three prosthetics have been performed at spread occlusal-stenotic lesions of BCT, carotid bifurcation resection with carotid arterial revascularization by prosthetics, carotic-subclavian autovenal bypass surgery, endarterectomy of BCT and endarterectomy of CCA, endarterectomy popliteal artery and subclavian-carotic bypass grafting. For seven patients, revascularization of the aorta-femoral zone was performed 4-6 days after the restoration of surgical intervention on the neck arteries.

In all cases, the restoration of blood flow in the aorta/iliac-femoral zone was performed only by aorto-bifemoral aloshtung. The main purpose of the given surgical intervention is to prevent a one-step delivery of oxygenated blood to the peripheral arterial bed and thereby to reduce the active phase of reperfusion-reoxygenative syndrome development.

In 22 out of 37 patients who had an occlusive process of aorto/iliac-femoral segment combined with femoral-distal occlusion, on the third days after the operation during the ultrasound examination revealed that the peak systolic velocity (PSV) and index of resistance (IR) at the level of the deep thoracic artery reached the level respectively, 23.2 - 1.5 cm/sec and 0.41 - 0.29 SU, and PSV and IR at the level of the tibioperinal trunk, respectively, 17.1 - 3.1 cm/sec and 0, 39 - 0.11 SU. The results obtained are lower, respectively, in 1.9 ($p < 0,05$) and 1,5 ($p < 0,05$) times the indicators that were on the first day of the postoperative period. This is due to blocking the occlusive process of the femoral-poplite segment of the anastomosis of the deep thoracic artery with the lowering artery of the knee and the popliteal artery, the arteries of the shin. These circumstances were indications for a hip-distal reconstruction, which was done in 22 observations on the third day after conducting the restoration operations on the aorto/ilac-femoral segments.

The decision of the order of restorative operations on brachiocephalic arteries and the aorto/ilac-femoral segment is based on the analysis of the localization of the occlusive process in both arterial basins, their hemodynamic characteristics, the degree of ischemia, and the tolerance of the brain to hypoxia [3]. Patients with atherosclerotic occlusion of the aorta/iliac-femoral arterial bed in conditions of chronic arterial insufficiency of lower limbs of IIIA-B - IVst. (according to the classification of Fontaine R. taking into account the criteria of the European Working Group (1992) have a high risk of developing reperfusion-reoxygenative complications [5]. Given the above, the revascularization of both arterial pools should be done in stages: the reconstruction of extracranial arteries is carried out initially, and after 4-6 days after the first surgical intervention – the revascularization of the aorta/iliac-femoral arterial region or revascularization of both arterial pools simultaneously.

Analyzing the results of revascularization operations at combined occlusive-stenotic lesion of extracranial and aortic/iliac-femoral arterial basins under conditions of high risk of development of reperfusion-reoxygenative syndrome, it was established that due to the differentiated choice of surgical intervention priority in both arterial zones, it was possible to prevent the development of such complications as progression of ischemic disorders of cerebrovascular blood flow, deepening of ischemia of the lower extremities, severe renal failure and some other dangerous reperfusion complications. Along with the above, at the intraoperative stage and in the early postoperative period, we diagnosed one manifestation of transient ischemic attack, 3 cases of rhythm disturbance and conduction of the cardiac muscle and 4 manifestations of transient renal failure.

The delayed revascularization of the femoral-distal arterial bed up to three days after aortic/iliac-bifemoral arterial bed has a number of positive features: besides restoring a complete blood supply to the distal lower limb, prevents a sudden oxygen shock on the peripheral arterial system of the lower limb and thereby reduces the activity of reperfusion-reoxygenative syndrome.

Conclusions. Staging and one-stage restorative operations in patients with combined atherosclerotic lesions of the extracra-

nial and aortic/iliac-femoral arterial beds in conditions of high risk of reperfusion-reoxygenative syndrome prevents a number of complications: development of acute ischemic disorders of cerebral blood circulation, deepening of ischemia of the lower extremities, severe renal failure and some other formidable reperfusion complications.

Compliance with Ethics Requirements. The authors declare that all the procedures and experiments of this study respect the ethical standards in the Helsinki Declaration of 1975, as revised in 2008(5), as well as the national law. Informed consent was obtained from all the patients included in the study.

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SUMMARY

SURGICAL TREATMENT OF COMBINED OCCLUSIVE-STENOTIC LESIONS OF EXTRACRANIAL ARTERIES AND AORTO/ILIAC-FEMORAL SEGMENT IN CONDITIONS OF HIGH RISK OF DEVELOPMENT OF REPERFUSION-REOXYGENATIVE COMPLICATIONS

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Today, the issues of surgical tactics remain unresolved, namely, the choice of volume and area of primary reconstruction with occlusion-stenotic lesions of extracranial arteries and aorto-iliac-femoral segment in conditions of high risk of reperfusion-reoxygenative syndrome.

The aim of the study - To improve the results of surgical treatment of combined occlusion-stenotic lesions of extracranial arteries and the aorto-iliac-femoral segment in conditions of high risk of development of reperfusion-reoxygenative complications.

The study included 58 patients with combined atherosclerotic lesions of extracranial arteries and aorto-iliac-femoral zone. To diagnose the nature of the lesion of the arterial bed and the features of hemodynamic disorders, ultrasound dopplerography, duplex scanning, X-ray contrast digital angiography were used. The proposed set of measures to prevent the development of reperfusion-reoxygenative complications was introduced to the system of preoperative preparation.

The decision of the order of restorative operations on the brachiocephalic arteries and the aorto-iliac-femoral segment was based on the analysis of the localization of the occlusive process in both arterial basins, their hemodynamic characteristics, the degree of ischemia and the tolerance of the brain to ischemia.

Due to the spread occlusion-stenotic lesion of extracranial arteries in combination with the stenotic process of the contralateral ICA in the stage of relative compensation of cerebral bloodflow in 7 patients, primarily the surgical intervention on the vessels of the neck was performed. Four to six days after the first surgical intervention, the revascularization of aorta/iliac-femoral segment was performed.

In 51 patients, one-time surgical intervention was performed on extracranial arteries and aorto-iliac-femoral basin.

Analyzing the results of revascularization operations, it can be argued that a differentiated approach to choosing the tactics of surgical treatment of multifocal atherosclerotic lesions of arterial basins should be used.

The revascularization of both arterial basins should be carried out in stages: a reconstructive operation on the extracranial arteries is performed initially, and 4 to 6 days after the first surgical intervention, the aorta/iliac-femoral arterial zone revascularization or revascularization of both arterial basins simultaneously.

Keywords: obliterative atherosclerosis, extracranial arteries, revascularization.

РЕЗЮМЕ

ХИРУРГИЧЕСКОЕ ЛЕЧЕНИЕ СОЧЕТАННОГО ОККЛЮЗИОННО-СТЕНОТИЧЕСКОГО ПОРАЖЕНИЯ ЭКСТРАКРАНИАЛЬНЫХ АРТЕРИЙ И АОРТО-ПОДВЗДОШНО-БЕДРЕННОГО СЕГМЕНТА В УСЛОВИЯХ ВЫСОКОГО РИСКА РАЗВИТИЯ РЕПЕРFUЗИОННО-РЕОКСИГЕНАЦИОННЫХ ОСЛОЖНЕНИЙ

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Некоторые вопросы хирургической тактики, в частности, выбор объема и зоны первичной реконструкции при окклюзионно-стенотическом поражении экстракраниальных артерий и аорто-подвздошно-бедренного сегмента в условиях высокого риска развития реперфузионно-реоксигенационного синдрома по сей день остаются нерешенными.

Цель исследования - улучшить результаты хирургического лечения сочетанного окклюзионно-стенотического поражения экстракраниальных артерий и аорто-подвздошно-бедренного сегмента в условиях высокого риска развития реперфузионно-реоксигенационных осложнений.

Обследовано 58 пациентов с сочетанным атеросклеротическим поражением экстракраниальных артерий и аорто-подвздошно-бедренного сегмента. Для диагностики характера поражения артериального русла и особенностей гемодинамических нарушений применяли ультразвуковую доплерографию, дуплексное сканирование, рентгеноконтрастную цифровую ангиографию. В системе предоперационной подготовки применен предложенный комплекс мероприятий для профилактики развития реперфузионно-реоксигенационных осложнений.

Решение очередности восстановительных операций на брахиоцефальных артериях и аорто-подвздошно-бедренном сегменте базировалось на анализе локализации окклюзионного процесса в обоих артериальных бассейнах, их гемодинамической характеристике, степени ишемии и толерантности головного мозга к ишемии.

В связи с распространенным окклюзионно-стенотическим поражением экстракраниальных артерий в сочетании со stenotическим процессом контралатеральной внутренней сонной артерии в стадии относительной компенсации мозгового кровообращения 7 пациентам проведены оперативные вмешательства на сосудах шеи. Спустя 4-6 дней после первого хирургического вмешательства осуществлена реваскуляризация аорто-подвздошно-бедренного сегмента.

51 пациенту проведено одномоментное хирургическое вмешательство на экстракраниальных артериях и аорто-подвздошно-бедренном бассейне.

Анализ результатов операций реваскуляризации показал, что к выбору тактики хирургического лечения мультифокального атеросклеротического поражения артериальных бассейнов следует применять дифференцированный подход.

Реваскуляризацию обоих артериальных бассейнов необходимо проводить поэтапно: первый этап - реконструк-

тивная операция на экстракраниальных артериях, второй этап - спустя 4-6 суток после первого хирургического вмешательства – реваскуляризация аорто-подвздошно-бедренной артериальной зоны или обеих артериальных бассейнов одновременно.

რეზიუმე

ექსტრაკრანიული არტერიების და აორტულ-თეძოსქვეშა-ბარძაყის სევმენტის შერწყმული ოკლუზიურ-სტენოზური დაზიანების ქირურგიული მკურნალობა რეპერფუზიულ-რეოქსიგენაციური გართულებების განვითარების მაღალი რისკის პირობებში

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კვლევის მიზანს წარმოადგენდა ექსტრაკრანიული არტერიების და აორტულ-თეძოსქვეშა-ბარძაყის სევმენტის შერწყმული ოკლუზიურ-სტენოზური დაზიანების ქირურგიული მკურნალობის შედეგების გაუმჯობესება რეპერფუზიულ-რეოქსიგენაციური გართულებების განვითარების მაღალი რისკის პირობებში.

გამოკვლეულია 58 პაციენტი ექსტრაკრანიული არ-

ტერიების და აორტულ-თეძოსქვეშა-ბარძაყის სევმენტის შერწყმული ათეროსკლეროზული დაზიანებით. არტერიული ნაკადის დაზიანების ხასიათის და ჰემოდინამიკური დარღვევების თავისებურებების დიაგნოსტიკისათვის გამოყენებულია ულტრაბგერითი დოპლეროგრაფია, დუპლექსური სკანირება, რენტგენოკონტრასტული ციფრული ანგიოგრაფია. წინასაოპერციო მზადების სისტემაში რეპერფუზიულ-რეოქსიგენაციური გართულებების განვითარების პროფილაქტიკისათვის გამოყენებულია შეთავაზებულ ღონისძიებათა კომპლექსი.

ექსტრაკრანიული არტერიების ოკლუზიურ-სტენოზური დაზიანებისა და კონტრალტერალური შიგნითა საძილე არტერიის სტენოზური პროცესის შერწყმის ხშირ გავრცელებასთან დაკავშირებით, თავის ტვინის სისხლის მიმოქცევის შედარებითი კომპენსაციის სტადიაზე 7 პაციენტს ოპერაცია ჩაუტარდა კისრის სისხლძარღვებზე. პირველი ქირურგიული ჩარევიდან 4-6 დღის შემდეგ ჩატარდა აორტულ-თეძოსქვეშა-ბარძაყის სევმენტის რევასკულარიზაცია.

51 პაციენტს ჩაუტარდა ერთმომენტიანი ქირურგიული ჩარევა ექსტრაკრანიულ არტერიებსა და აორტულ-თეძოსქვეშა-ბარძაყის აუზზე.

ორივე არტერიული აუზის რევასკულარიზაცია ჩატარდა ეტაპობრივად: პირველი ეტაპი – რეკონსტრუქციული ოპერაცია ექსტრაკრანიულ არტერიებზე, მეორე ეტაპი – პირველი ქირურგიული ჩარევიდან 4-6 დღის შემდეგ - აორტულ-თეძოსქვეშა-ბარძაყის არტერიული ზონის, ან ორივე არტერიული აუზის ერთმომენტიანი რევასკულარიზაცია.

ANTIBACTERIAL THERAPY FOR PURULENT-SEPTIC COMPLICATIONS IN PATIENTS WITH COMBAT RELATED PENETRATING CRANIOCEREBRAL GUNSHOT WOUNDS

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Penetrating craniocerebral gunshot wounds (PCGW) are a threat to victims' lives and long-term health, primarily due to the injury and resulting destruction of brain tissue as well as bacterial penetration of detritus into the cranial cavity and subsequent risk of wound infection. Bacterial contamination is already known to occur in cases of soft tissue injuries of the head and increases considerably if a non-sterile injuring projectile penetrates the cranial cavity [6]. Following the injuring projectile, fragments of skull, skin, hair, cranial bones, hot air, powder gases, etc., pierce through the brain, significantly increasing the risk of intracranial purulent-septic complications (PSCs) [1,18].

According to the U.S. military guidelines on the prevention of infections associated with combat-related injuries [8], cefazolin (2 g every 6-8 h) is prescribed as initial empiric antibiotic therapy

(ABT) for PCGWs, and if wound contamination is significant, metronidazole (500 mg every 8–12 h) is added. Clindamycin (1,800–2,700 mg/day) or ceftriaxone (2-4 g/day) and metronidazole (500 mg every 8-12 h) are considered to be therapeutic alternatives. In cases of penicillin allergy, vancomycin (2 g/day) and ciprofloxacin (400–800 mg/day) are recommended.

Intravenous antibiotics should be administered as soon as possible after the injury, preferably within 3 h. The antibiotic used should be sufficient to manage pathogenic microorganisms that can contaminate wounds during injury, including normal skin and intestinal flora, such as *Staphylococcus*, *Escherichia coli* and anaerobes of the gastrointestinal tract.

Initial antibacterial therapy should not target pathogens with multiple antibiotic resistance such as *Acinetobacter baumannii*,

Pseudomonas aeruginosa or *Klebsiella pneumoniae*. Because of decreased levels of methicillin-resistant *Staphylococcus aureus* infections and clinical evidence that adequate wound drainage, rather than antibiotics, is considered primary treatment in cases of abscesses, empiric therapy with vancomycin or linezolid may not be appropriate. Data have shown that empiric use of broad-spectrum antibiotics can lead to subsequent antibiotic-resistant infections [10].

The duration of ABT should be reduced to a minimum. Studies have reported that long-term therapy diminishes the outcome [15]. Wounds should be cleaned thoroughly at each bandage change with 4% chlorhexidine gluconate. Treatment of gunshot wounds often involves delayed initial closure of extremity wounds. However, facial and cerebral injuries require early primary closure of mucous membranes or dura mater to reduce the incidence of infections and aesthetic complications [4,18].

Study objective - to analyse pathogens and their susceptibility to antibiotic therapy in combat-related penetrating craniocerebral gunshot wounds patients and establish recommendations for the treatment of post-traumatic meningoencephalitis.

Material and methods. One hundred twenty-one patients who were admitted to the Public Institution, Mechnikov Dnipropetrovsk Regional Clinical Hospital, Dnipro, Ukraine, from 25 May 2014, to 31 December 2017, were successively enrolled in the study. The main inclusion criterion was a combat-related PCGW. All wounds were sustained during the combat operations in the eastern Ukraine.

Penetrating craniocerebral wounds (PCWs) are injuries that damage the integrity of the dura mater, which can lead to bacterial penetration into the skull. Clinical symptoms of PCWs include discharge of cerebrospinal fluid (CSF) and/or brain fragments from the wound. Symptoms of a penetrating craniocerebral wound on head and neck computed tomography include intradural or intracerebral foreign bodies and pneumocephalus [17].

ABT was administered according to the guidelines for the treatment of combat-related injuries [8]. Empirical ABT lasted for up to 5 days. Antibacterial therapy was adjusted according to results of bacteriological examination, and its efficacy was based on procalcitonin, a generally recognised marker for sepsis. Microbiological studies were performed in a clinicodiagnostic laboratory with a bacteriological analyser, VITEK 2 (bio-

Mérieux., Inc., France). We were able to identify the pathogen and prescribe targeted ABT (etiotropic treatment) in most cases of PSCs.

Results and their discussion. The study included 121 male patients aged 18 to 56 years (mean, 34.1±9.1). The Glasgow Coma Scale score ranged from 3 to 15 (mean, 10±4), and the Injury Severity Score ranged from 16 to 57 (mean, 27.7±7.6). Overall, 101 patients (83.5%) were diagnosed with shrapnel wounds and 20 (16.5%) with gunshot wounds.

The characteristics of microorganisms extracted during microbiological study of patients with PSCs are shown in Table 1.

Bacteriological examination of patients with PSCs due to penetrating CGSWs revealed that *Acinetobacter baumannii*, one of the most important representatives of so-called hospital flora, caused the highest incidence of infections, including post-traumatic meningitis. *A. baumannii* typically involves the skin and respiratory tract but can also cause other infections due to translocation, including those related to past medical procedures.

The most notable characteristic of this microorganism is its high pathogenicity in hospital patients who have been seriously ill for an extended period, including those with a weakened immune system. Patients with invasive medical devices, such as vascular and other catheters, surgical sutures or respiratory ventilators, and those who have received renal replacement therapy (haemodialysis) or antimicrobial therapy for the last 90 days are also at risk of developing *A. baumannii* infections.

Data on the relationship between the high incidence of this pathogen in patients with combat-related gunshot wounds have been reported. *A. baumannii* became particularly well known during the U.S. Army's military operations in Iraq during Operation Iraqi Freedom, causing an increased incidence of bloodstream infections among U.S. military personnel, called Iraqibacter [9].

A. baumannii is more likely to affect moist tissues, such as mucous membranes or damaged skin areas, where it may cause necrotising infection. Areas of infection or colonisation may involve the respiratory tract, blood, pleural fluid, urinary tract, surgical wounds, central nervous system (CNS), skin and eyes. Pneumonia can pose a threat to patients requiring mechanical ventilation, as *A. baumannii* has the ability to produce biofilms on endotracheal tube surfaces. A study conducted in 2003 on board the U.S. Navy hospital ship *Comfort*, which provided

Table 1. Results of bacteriological examinations

Microorganism	Infection location				
	CSF	Wound infection	Respiratory tract infection	Urinary tract infection	TOTAL
<i>Acinetobacter baumannii</i>	5	1	5	0	11
<i>Klebsiella pneumoniae</i>	2	1	4	0	7
<i>Escherichia coli</i>	0	1	1	0	2
<i>Pseudomonas aeruginosa</i>	1	2	5	0	8
<i>Staphylococcus aureus</i>	0	0	1	0	1
<i>Staphylococcus epidermidis</i>	2	0	0	0	2
<i>Staphylococcus haemolyticus</i>	0	0	3	0	3
<i>Enterobacter agglomerans</i>	1	0	2	0	3
<i>Enterococcus faecalis</i>	1	1	0	0	2
<i>Enterococcus faecium</i>	0	0	1	0	1
<i>Candida</i>	0	0	0	1	1
TOTAL	12	6	22	1	41

emergency medical care to injured U.S. military personnel in the Persian Gulf, showed that 4.1% of all skin and soft tissue infections were associated with *A. baumannii* [5].

The incidence of *A. baumannii* infections is increasing, especially in the U.K. and the U.S., as coalition forces exposed to bacteria in field hospitals return home to recover and rehabilitate.

Together with the development of skin, soft tissue, respiratory tract and bloodstream infections due to *A. baumannii*, neurosurgical patients are reported to have worsening of nosocomial post-surgical meningitis, which is a serious problem in those undergoing intensive therapy [3,12].

The second most common pathogen in our patients was *Pseudomonas aeruginosa*, which is also characteristic of hospital-acquired postoperative wound, respiratory tract and bloodstream infections in patients with a weakened immune system and those requiring invasive medical devices.

The efficacy of infection control measures, particularly hand hygiene and disinfection of the surrounding area, plays a major role in the spread of *P. aeruginosa*. Antibiotic-resistant *Pseudomonas* can be fatal in critically ill patients. In total, 51,000 medically related *P. aeruginosa* infections are estimated to occur each year in the United States. More than 6,000 (13%) cases have multiple drug resistance, with approximately 400 deaths associated with these infections per year [2].

Meningitis and ventriculitis caused by *P. aeruginosa* are primarily nosocomial infections and are related to previous neurosurgical procedures. Statistics have shown that *P. aeruginosa* is responsible for 1%–18% of cases of nosocomial meningitis [14].

K. pneumoniae was the third most common pathogen with infectious complications in our patients. In cases of *K. pneumoniae*, meningitis is most often considered a hospital-acquired infection related to neurosurgery and is associated with high mortality, despite the wide availability of antimicrobial and adjunctive therapies, due to its abnormal characteristics and the difficulty in detecting it at an early stage. Clinical outcomes of *K. pneumoniae* meningitis are often unsatisfactory. Studies have reported higher rates of diabetes, alcoholism and chronic liver diseases in addition to pre-existing infections (such as pyogenic liver abscess, septic endophthalmitis, pneumonia, otitis media, urinary tract infection, lumbar discitis and perianal abscess) in patients with *K. pneumoniae* meningitis who had undergone neurosurgical procedures [11].

Staphylococcus epidermidis are typically in hospital infections associated with the use of intravenous catheters and permanent prostheses. Colonisation of *S. epidermidis* on the skin of patients and health care workers is considered the most common source of these infections. This pathogen's ability to stick and form biofilms on the surfaces of foreign bodies is considered to be its most important mechanism. *S. epidermidis* meningitis is typically associated with neurosurgical procedures and devices, such as ventriculoperitoneal shunts, as well as head injury [13].

Meningitis caused by *Enterobacter* species rarely occurs in adults, although it may develop in neurosurgery patients and those sustaining neurotrauma. Treatment is often complicated because of the resistance of many *Enterobacter* isolates to third-generation cephalosporins and poor penetration of other antibiotics into the CNS. The most common pathogens are *Enterobacter cloacae*, *Enterobacter aerogenes* and *Enterobacter agglomerans* (50%, 34% and 16% of cultures, respectively). Overall, 47% of patients had clinical recovery/improvement, whereas 21% died [7].

Enterococcus faecalis caused by meningitis is an uncommon disease accounting for <1% of all cases of adult meningitis. This infection typically affects patients with immune disorders or CNS injuries, primarily in the form of hospital-acquired infections. It is also associated with neurosurgical procedures and shunting. The overall mortality rate was 21% in the present study. Adverse outcomes largely correspond with old age, presence of serious underlying disease, associated enterococcal infection, bacteraemia, septic shock and absence of fever on admission [16].

Thus, our careful analysis of infectious complications of pathogens in patients with PCWs revealed that most cases are nosocomial infections. The most effective prevention strategy includes a modern infection control system focusing on barrier measures to prevent the spread of infection. Targeted ABT toward the pathogen's sensitivity to antibiotics and antiseptics is the preferred treatment strategy.

We performed a detailed analysis of the sensitivity of microorganisms extracted from biological tissues to antibacterial agents (Table 2).

Sensitivity analysis of microorganisms detected in our patients to antibiotics revealed that, in most cases, the efficacy of the first-line ABT (ACCESS) was low and it was often necessary to prescribe broad-spectrum antibacterial drugs, including those related to second-line antibiotics (WATCH) and reserve drugs (RESERVE), according to the World Health Organisation classification [20].

Data in the literature confirm the results of our bacteriological studies.

Modern *A. baumannii* infections are characterised by multi-drug resistance to antimicrobials and antiseptics. The MYSTIC study, performed in 48 European hospitals between 2002 and 2004, reported that only 73.1% of *A. baumannii* isolates were susceptible to meropenem and 69.8% to imipenem. Susceptibility to other antibiotics was also extremely low: 32.4%, 34.0% and 47.6% were sensitive to ceftazidime, ciprofloxacin and gentamicin, respectively. Despite extensive administration of broad-spectrum ABT, the mortality rate in such infections can reach 70% [19].

The high resistance of *P. aeruginosa* infections requires treatment with intravenous antibiotics such as ceftazidime, carbapenems (meropenem and imipenem), aminoglycosides (gentamicin, amikacin or tobramycin) and ciprofloxacin, often in combination with intrathecal agents such as aminoglycosides or colistin. The recommended duration of therapy is from 14 to 28 days. In spite of this, treatment failures and relapses occur, with mortality reaching 80% [14].

For the treatment of *K. pneumoniae* infections in patients with CNS injury undergoing neurosurgical procedures, the primary component of survival, recovery and a favourable neurological outcome is early intravenous administration of first-line antibiotics before decline in neurological status (GCS≤7). Extended-spectrum cephalosporins are typically the drugs of choice, but aztreonam, carbapenems, aminoglycosides and ciprofloxacin are also used with varying degrees of effectiveness. Expected therapy duration ranges from 14 to 21 days [11].

In the present study, intracranial PSCs were detected in 14 (11.6%) patients. In addition, isolated meningoencephalitis occurred in eight patients, meningoencephalitis combined with ventriculitis in three and meningoencephalitis combined with ventriculitis and subdural empyema in two. Recurrent meningoencephalitis was complicated by multiple brain abscesses in one patient.

Table 2. Detected microorganism sensitivity to antibiotics

Microorganism	Sensitivity to antibiotics			
	CSF	Wound infection	Respiratory tract infection	Urinary tract infection
<i>Acinetobacter baumannii</i>	Cefoperazone/sulbactam, Meropenem, Tigecycline, Tobramycin	Tobramycin	Colistin, Tigecycline, Cefoperazone/sulbactam	-
<i>Klebsiella pneumoniae</i>	Amikacin, Piperacillin/tazobactam, Tigecycline, meropenem	Tobramycin	Amikacin, Cefoperazone/sulbactam, Meropenem, Gentamicin, Tobramycin, Trimethoprim/sulfamethoxazole, Ciprofloxacin	-
<i>Escherichia coli</i>	-	Gentamicin, Amikacin, Meropenem, Tobramycin, Ciprofloxacin	Meropenem, cefoperazone/sulbactam, Ceftriaxone	-
<i>Pseudomonas aeruginosa</i>	-	Cefoperazone/sulbactam, Gentamicin, Amikacin, Meropenem, Ciprofloxacin, Colistin, Ceftazidime	Cefoperazone, cefoperazone/sulbactam, Tigecycline, Tobramycin, Colistin, Levomycetin	-
<i>Staphylococcus aureus</i>	-	-	Vancomycin, Tigecycline, Gentamicin	-
<i>Staphylococcus epidermidis</i>	Levofloxacin, Tigecycline, Trimethoprim	-	-	-
<i>Staphylococcus haemolyticus</i>	-	-	Levofloxacin, Tigecycline, Cefoperazone/sulbactam, Clindamycin	-
<i>Enterobacter agglomerans</i>	Amikacin, Meropenem	-	Tigecycline, Meropenem	-
<i>Enterococcus faecalis</i>	Linezolid, Vancomycin	-	Imipenem, Colistin, Cefoperazone/sulbactam, Tobramycin	-
<i>Enterococcus faecium</i>	-	-	Amphotericin B, Caspofungin	-
<i>Candida</i>	-	-	-	Fluconazole, Voriconazole

Post-traumatic meningoencephalitis caused by *S. epidermidis* is characteristically associated with neurosurgical procedures and devices, such as ventriculoperitoneal shunts, as well as head injury. Glycopeptides (vancomycin and teicoplanin) are usually effective for intensive therapy, and oxazolidinones (linezolid) are effective in treating methicillin-resistant strains [13].

Treatment of meningoencephalitis caused by *Enterobacter* family microorganisms is characterised by low resistance to third-generation cephalosporins. Successful therapy involves intravenous and intrathecal administration of aminoglycosides, trimethoprim-sulfamethoxazole, piperacillin and ciprofloxacin [7].

Treatment of meningoencephalitis associated with *Enterococcus faecalis* usually includes ampicillin, penicillin, vancomycin and linezolid, with or without aminoglycosides, for an average period of 18 days. Other effective agents are intrathecal gentamicin and intravenous dexamethasone [16].

On the basis of the present study, we have formulated recommendations for antibiotic treatment of post-traumatic meningoencephalitis in combat-related CGSWs (Table 3).

Thus, because of the high risk of multi-drug-resistant hospital flora translocation, with clinical laboratory data indicating development of post-traumatic meningoencephalitis, we started de-escalation of empiric ABT with the broadest-spectrum drugs as monotherapy, and combination therapy was primarily used in cases of expected gram-positive and gram-negative aerobic and anaerobic pathogenic bacteria. Modification of empiric ABT was performed on the basis of clinical data regarding development of the infection process over time, SIRS markers, sepsis and multisystem failure. After receiving results of microbiological studies, targeted medication of ABT was initiated immediately, with a preference for etiotropic therapy and aggressive microbiological control.

Conclusions. Modern combat-related GSWs are serious injuries with numerous entry points for infection. Factors leading to immunosuppression in patients and promoting generalised infection of gunshot wounds include the presence of multiple primary wound infections, blood loss, anaemia, shock, malnutrition and chronic stress.

Table 3. Post-traumatic meningoencephalitis ABT for craniocerebral gunshot wounds

ABT					Microorganism	
Empiric in escalation mode De-escalation	Empiric de-escalation modified (when there is no culture growth)	Targeted, initial (according to inoculation results) Etiotropic-1	Targeted, modified for the 1 st time (according to inoculation results) Etiotropic-2	Targeted, modified for the 2 nd time (according to inoculation results) Etiotropic-2		
Meropenem+metronidazole (3) Meropenem-vancomycin (6) Meropenem-colimycine (1) Meropenem-ornidazole (1) Meropenem-cefosulbin Cefoperazone/sulbactam-ertapenem, Tienam-ornizol	Cefoperazone/sulbactam, Vancomycin-meropenem, Tienam-vancomycin, Vancomycin-tigecycline, Tigecycline-piperacillin/tazobactam-linezolid, Tigecycline-linezolid-colomycin, Linezolid-colomycin Tigecycline, Levofloxacin-vancomycin	Ceftriaxone/sulbactam, Colomycin, Piperacillin/tazobactam, Cefoperazone/sulbactam, Tigecycline, Biseptol,	Tigecycline	+Tigecycline, etc.	<i>Acinetobacter baumannii</i>	
		Tigecycline		Gentamicin	<i>Klebsiella pneumoniae</i>	
		Meropenem (2)				<i>Escherichia coli</i>
			Tigecycline	Chloramphenicol		<i>Pseudomonas aeruginosa</i>
		Vancomycin				<i>Staphylococcus aureus</i>
		Biseptol, vancomycin	Tigecycline			<i>S. epidermidis</i>
			Tigecycline (2)			<i>S. haemolyticus</i>
						<i>Enterobacter agglomerans</i>
			Vancomycin		Linezolid	<i>Escherichia faecalis</i>
				<i>Escherichia faecium</i>		
	Fluconazole			<i>Candida</i>		

ABT for severe PSCs of PCGWs is effective when thoroughly and continuously controlled (daily or every 2–3 days) by bacteriological monitoring, a so-called monitoring-based antibiotic therapy.

Initial elimination of pathogens is often accompanied by superinfection development with ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacter* species), and use of procalcitonin-guided ABT is recommended. Procalcitonin level <0.5 ng/mL is a sign to stop ABT.

It is reasonable to use combined ABT, combining drugs with different mechanisms of action, and it is acceptable to monitor the mode of dose- and time-dependent antibiotics administration.

In order to achieve minimum inhibitory (suppressive) concentration of an antibacterial drug in cases of increased clearance, the maximum permitted dose of the drug should be used. Increased drug clearance may be due to increased renal blood flow, hypervolaemia, overhydration or hyperdynamic circulation.

Conclusions.

Regarding PSC pathogens: Our results showed that, in most cases, such infections are considered nosocomial and are related to invasive medical procedures and devices. The most effective strategy in their prevention is implementing a modern infection control system with an emphasis on barrier measures against the spread of infection. Targeted ABT with early identification of the pathogen and its sensitivity to antibiotics and antiseptics is the preferred treatment strategy.

Regarding AB sensitivity of PSC pathogens: Our results showed that the efficacy of first-line antibiotics (ACCESS) was typically low and it was often necessary to prescribe broad-spectrum antibiotics, including those related to second-line antibiotics (WATCH) and reserve drugs (RESERVE), according to the WHO classification.

Regarding ABT of PSC: The use of initial de-escalation of empiric ABT using the broadest-spectrum drugs, mainly as a part of combination therapy for expected gram-positive and gram-negative aerobic and anaerobic infection pathogens, is recommended. Modification of empiric ABT is made on the basis of clinical

data regarding development of the infectious process over time, SIRS markers, sepsis and multiple organ dysfunction. Targeted modification of ABT is performed immediately upon determining the results of microbiological studies, with a preference for etiotropic therapy and aggressive microbiological control.

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SUMMARY

ANTIBACTERIAL THERAPY FOR PURULENT-SEPTIC COMPLICATIONS IN PATIENTS WITH COMBAT RELATED PENETRATING CRANIOCEREBRAL GUNSHOT WOUNDS

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Aim - to evaluate pathogens and their susceptibility to antibi-
otic therapy (ABT) in combat-related penetrating craniocerebral
gunshot wound (PCGW) patients and develop recommendations
for treatment of post-traumatic meningoencephalitis.

We conducted a prospective analysis of examination and
treatment results of 121 patients who were admitted to the Pub-
lic Institution, Mechnikov Dnipropetrovsk Regional Clinical
Hospital, Dnipro, Ukraine, from 25 May 2014, to 31 December
2017, and were successively enrolled in the study. Intracranial
purulent-septic complications were diagnosed in 14 (11.6%)
patients including eight cases of isolated meningoencephalitis,
three cases of meningoencephalitis combined with ventriculitis,
two cases of meningoencephalitis combined with ventriculitis
and subdural empyema and one case of multiple brain abscesses.

In most cases of combat-related craniocerebral wounds, infec-
tions are considered nosocomial and typically related to medi-
cal procedures and devices. In most cases, the effectiveness of
first-line antibiotics was low, and it was often necessary to pre-
scribe broad-spectrum ABT, including those related to second-
line antibiotics and reserve drugs, according to the World Health
Organisation classification. The use of initial de-escalation of
empiric ABT with the broadest-spectrum drugs, mainly as a part
of combination therapy for expected gram-positive and gram-
negative aerobic and anaerobic infection pathogens, is recom-
mended.

Keywords: purulent-septic complications, pathogens, sus-
ceptibility of pathogens, antibacterial therapy, penetrating cra-
niocerebral wounds, combat-related gunshot wounds.

РЕЗЮМЕ

АНТИБАКТЕРИАЛЬНАЯ ТЕРАПИЯ ГНОЙНО-СЕПТИЧЕСКИХ ОСЛОЖНЕНИЙ У ПАЦИЕНТОВ С БОЕВЫМИ ОГНЕСТРЕЛЬНЫМИ ПРОНИКАЮЩИМИ ЧЕРЕПНО-МОЗГОВЫМИ РАНЕНИЯМИ

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Цель исследования - анализ возбудителей инфекций и их чувствительности к антибактериальной терапии у пациентов с боевыми огнестрельными проникающими черепно-мозговыми ранениями; разработка рекомендаций по лечению посттравматического менингоэнцефалита.

Проведен проспективный анализ результатов обследования и лечения 121 пациента с боевыми проникающими черепно-мозговыми ранениями. Пострадавшие поступили в Днепропетровскую областную клиническую больницу им. И.И. Мечникова в период с 25 мая 2014 г. по 31 декабря 2017 г. Внутрочерепные гнойно-септические осложнения выявлены у 14 (11,6%) раненных: изолированный менингоэнцефалит - 8 случаев, менингоэнцефалит в сочетании с вентрикулитом - 3 случая, менингоэнцефалит в сочетании с вентрикулитом и субдуральной эмпиемой - 2 случая, множественное абсцедирование

вещества головного мозга - 1 случай. Инфекции у пациентов с боевыми черепно-мозговыми ранениями в большинстве случаев следует рассматривать как нозокомиальные, связанный с медицинскими процедурами и устройствами. Терапия антибиотиками первого выбора (ACCESS) в большинстве случаев оказалась неэффективной, часто возникала необходимость назначения антибактериальных препаратов широкого спектра действия, в том числе антибиотиков второго выбора (WATCH) и препаратов резерва (RESERVE) по классификации ВОЗ. В результате проведенного исследования доказано преимущество использования стартовой эмпирической дескалационной антибактериальной терапии препаратами максимально широкого спектра действия в режиме комбинированной терапии грам-положительных и грам-отрицательных аэробных и анаэробных возбудителей инфекций.

რეზიუმე

ჩირქოვან-სეპტიკური გართულებების ანტიბაქტერიული თერაპია პაციენტებში საბრძოლო ცეცხლნასროლი გამჭოლი ქალა-ტვინის ჭრილობებით

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¹სახელმწიფო დაწესებულება „უკრაინის ჯანდაცვის სამინისტროს დნეპროპეტროვსკის სამედიცინო აკადემია“; ²ი. მენჩიკოვის სახ. დნეპროპეტროვსკის საოლქო კლინიკური საავადმყოფო, უკრაინა

კვლევის მიზანს წარმოადგენდა ინფექციების გამომწვევების და მათი ანტიბაქტერიული თერაპიის მიმართ მგრძობელობის ანალიზი დაზარალებულებში ცეცხლნასროლი გამჭოლი ქალა-ტვინის ჭრილობებით, რეკომენდაციების შემუშავება ტრავმის შემდგომი მენინგოენცეფალიტის მკურნალობის თაობაზე.

გაანალიზებულია 121 საბრძოლო ცეცხლნასროლი გამჭოლი ქალა-ტვინის ჭრილობებით დაზარალებულის მკურნალობა ი. მენჩიკოვის სახელობის დნეპროპეტროვსკის საოლქო კლინიკური საავადმყოფოში, 2014 წლის 25 მაისიდან 2017 წლის 31 დეკემბრამდე. შიდაქალის ჩირქოვან-სეპტიკური გართულებები გამოუვლინდა 14 (11,6%) დაჭრილს: იზოლირებული მენინგოენცეფალიტი - 8 შემთხვევა, მენინგოენცეფალიტი ვენტრიკულიტთან ერთად - 3 შემთხვევა, მენინგოენცეფალიტი ვენტრიკულიტთან და სუბდურალურ ემპიემასთან ერთად - 2 შემთხვევა, თავის ტვინის ნივთიერების მრავლობითი აბსცედირება - 1 შემთხვევა.

ინფექციები დაზარალებულებში საბრძოლო ცეცხლნასროლი ქალა-ტვინის ჭრილობებით უმეტეს შემთხვევაში უნდა განვიხილოთ, როგორც ნოზოკომიალური, რომლებიც დაკავშირებულია სამედიცინო პროცედურებსა და მოწყობილობებთან. თერაპია პირველადი შერჩევის ანტიბიოტიკებით (ACCESS) უმეტეს შემთხვევაში არ იყო ეფექტური, ხშირად აუცილებელი ხდებოდა მოქმედების ფართო სპექტრის მქონე ანტიბაქტერიული პრეპარატების დანიშვნა, მათ შორის, რომლებიც განეკუთვნება მეორე შერჩევის ანტიბიოტიკებს (WATCH) და რეზერვის პრეპარატებს (RESERVE), მსოფლიო ჯანდაცვის ორგანიზაციის კლასიფიკაციის თანახმად. ჩვენებაშია სასტარტო ემპირიული დეესკალაციური ანტიბაქტერიული თერაპიის გამოყენება მოქმედების მაქსიმალურად ფართო სპექტრის პრეპარატებით, უპირატესად, კომბინირებული თერაპიის რეჟიმში სავარაუდო გრამ-დადებითი და გრამ-უარყოფითი აერობული და ანაერობული ინფექციების გამომწვევების მიმართულებით.

COMPLICATIONS OF TREATMENT BY TITANIUM ELASTIC INTRAMEDULLARY NAILS IN CHILDREN WITH FEMORAL SHAFT FRACTURES

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Treatment of pediatric shaft (diaphyseal) fracture of the femur is important subject in traumatology and orthopedics. Topicality of this subject is based on the frequency of pathology in pediatric population [1,7,14] and on the different methods of treatment. In 2013 Atsuko Nakaniida and colleagues [1] accounted that from all pediatric fractures which require hospitalization 20 % was femoral diaphyseal fractures. The same number - 21.7% - showed Galano and colleagues in their article [7] in 2005.

There are several methods for treatment of pediatric femoral shaft fractures, which can be divided by conservative and operative methods. Choice of treatment methods depends on type of fractures, doctor's personal opinion, hospital environment, child and parent's requests and etc.

Osteosynthesis by titanium elastic intramedullary nails (TEN) became very popular over the world in last 2 decades as a method of first choice for pediatric diaphyseal fractures, including femoral diaphyseal fractures.

Most of article's reports about Osteosynthesis by TEN have good results [3,5,9,10,23]. There are few complications, which mainly includes pain and irritation on nail insertion site, nail migration, shortening, loss of reduction, Malunion, nonunion, refracture [2,4,11,15,16,18,21,24,25,27]. There are discussions that all complications depend on objective circumstances or just on poor surgical technique and some subjective reasons which can be improved [2,11,15,18,24,27]. Most recent study of complications of fixation by TEN [20] shows that instead of improved knowledge and experience of this kind of treatment, there are still complications related to this method.

The goal of our study is to share our experience regarding the pediatric femoral shaft treatment by TEN, to show efficacy of method and low rate of unimportant complications, to demonstrate that treatment by TEN is excellent and first choice method of treatment of this pathology.

Material and methods. This is a retrospective cohort study. We reviewed Institutional Review Board approval before beginning the study. From 2013 to 2017 from the medical and X-ray records of „M Iashvili Children's Central Hospital“. we collected all data of patients with diagnosis of femoral shaft fractures and of those who were hospitalised for this reason. Inclusion criterias were patients from 2 to 15 years of age, with femoral shaft fracture who underwent operative treatment - osteosynthesis by titanium elastic nail (TEN). Exclusion criterias were politrauma patients, patients with open and pathological fractures, patients with metabolic bone disease and with neuromuscular disease. All surgical procedures were performed by same orthopedic team. Most of patient underwent second surgery to remove the implants. We collected data regarding patients age, sex, weight and mechanism of injury, types of fractures, operative procedure duration, radiographic and functional outcomes of treatment, early and late complications. For assesing the clinical and radiological outcome of treatment we used the scoring criteria for TEN by John M. Flynn et al. [11].

Results and their discussion. There were total of 188 patient in our institution during 2013-2017 with fracture of femoral shaft. From these patients inclusion criteria were met by 119 patients. From the rest of 69 patient there were 2 patients with

metabolic bone disease, 5 patients with open fractures, 7 patients with neuromascular disease, 7 pathological fractures and 48 patients with politrauma. There were 34 girls and 85 boys (ratio 1: 2,5) with mean age of 7 years (range 2 - 15). Patients' mean weight was 23 kg (range 10,5 to 61 kg). According to International Statistical Classification of Diseases and Related Health Problems 10th Revision (ICD-10 Version:2016) trauma mechanisms were 54 cases of fall on the same level from slipping, strapping and strumbling (W01), 22 cases of different types of traffic accidents (V43.6, V79.8, V46.3, V69.8, V47.6, V49.1, V43.6, V28.0), 19 cases of fall from, out of or through building or structure (W13), 15 - sports related injury and 9 -others. According to Muller AO classification of fractures there were fractures of type A1-52 cases, type A2- 29 cases, A3-26 cases; B1-5 cases; B2- 2 cases; B3- 1 cases; C1 - 3 cases and C3 - 1 case.

Duration of operation was an average of 50 minutes (range 25 - 100 minutes). In 5 cases open reduction and internal fixation were done. In rest of the 114 cases close reduction and internal fixation were done. The insertion of „end cup“ for elastic nails during the surgery was performed in 25 cases.

Average time for patients to stay in hospital was 3 days (from 2 to 7 days). First follow up of patients was seen between 4 to 8 weeks. Patients who were permitted began partial weight bearing (with crutches or with assistance) average for 5 week (range from 4 to 8 weeks). The average time for final follow up and admission in hospital for removing the implants was 9 months (range 3 - 17 month). According to the scoring criteria for TEN by Flynn et al. the results are listed in Table 1.

According to our study, in group of satisfactory results from 29 patients 17 patients had some degree of irritation of soft tissues caused by ends of nails; 7 patients had varus deformation of about 10 degrees and 5 patients resulted in shortening of femur from 1 to 2 cm. All the patients with soft tissue irritations were in the group which did not have „end cups“ on the nails - 18 % (17 patients from 94). No one had same complaint in group of 25 patients with insertion of nails „end cups“.

In group of poor results two patients had significant pain caused by ends of nails and they had procedure of removal of the implants before usual time - 3 months after the operation.

One patient with poor result was 14 year old boy who received femoral fracture (type B1) after traffic accident with weight of 55 kg. During the operation we inserted two 4 mm diameter elastic nails with appropriate technique . Immediately after operation X-ray showed satisfactory position of fracture (around 10 degrees of varus deformation). On follow up after 7 weeks of discharging from hospital we discovered varus angulation of nearly 20 degrees on X-ray. On this visit X-ray showed satisfactory healing and we allowed patient partial weight bearing with crutches for 2 weeks. We saw this patient 1 year after operation and there was varus angulation on x-ray again of about 20 degrees. Patients' gait pattern was changed and we offered him to continue some physiotherapy procedures. We discussed this complication with patient and his parents and offered them to do corrective osteotomy but they denied further treatment and after one year we removed the nails. Patient currently continues doing active sports. Below are two x-rays of this patient on picture 1 and 2.

Table 1. TEN outcome scoring

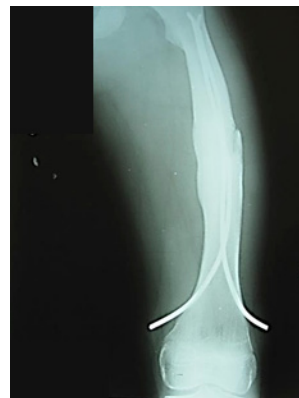
	Excellent results	Satisfactory results	Poor results
Leg length inequality	<1.0 cm	<2.0 cm	>2.0 cm
Malalignment	5 degrees	10 degrees	>10 degrees
Pain	non	non	present
Complication	non	minor and resolved	major complication and/or lasting morbidity
Patients results (n=119)	87	29	3

Let's mention that this patient was one of the five overweight patients. Second patient's weight was 55kg, third patient's weight - 61 kg, fourth patient's weight - 56 kg and the fifth patient's weight - 58 kg. For three patients also two 4 mm diameter elastic nails were used and for fifth patient (with weight - 58 kg) was used

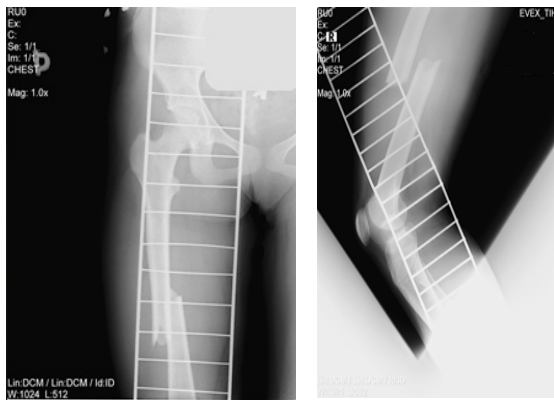
3 elastic nails - 4mm diameter - additional third nail was inserted from lateral side for correction of varus deformation of femur. All four patients had type A3 fractures. For all 4 overweight patients the results were excellent. Below are shown pre-op. and post-op. x-Ray's of some of these overweight children.



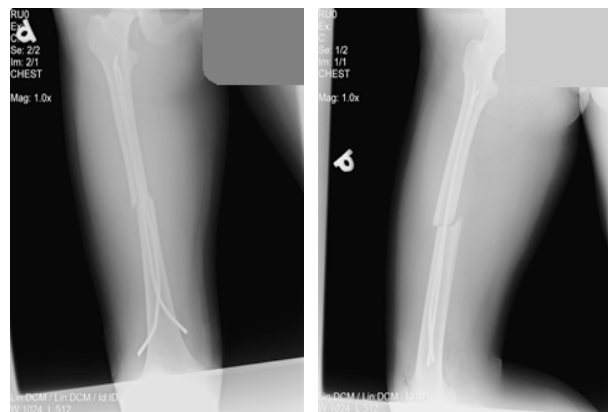
Pic. 1. 14 year old boy's x-ray after 7 weeks of operation



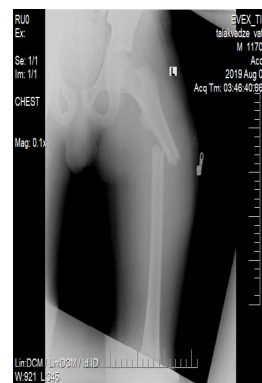
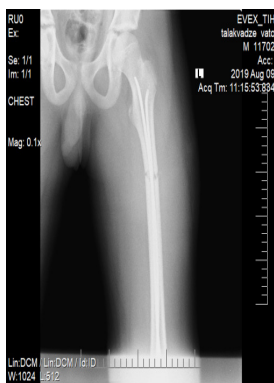
Pic. 2. X-ray after 1 year of follow up of same patient



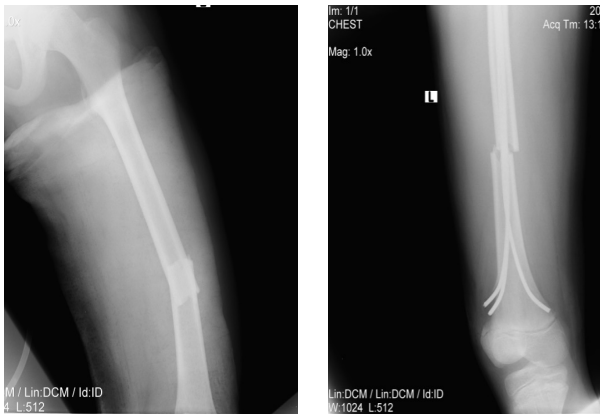
Pic. 3-4. Patient #1. 14 year old girl's x-ray after fracture (patient's weight is 61 kg)



Pic. 5-6. Patient #1. X-ray of same patient after operation



Pic. 7-8. Patient#2. X-rays of 14 years boy with femoral shaft transverse fracture (type A3) with weight 55 kg before and after operation



Pic. 9-10. Patient#3: X-rays of 15 years boy with femoral shaft transverse fracture (type A3) with weight 58 kg before and after operation

There was not a single case of early or late infection and no case of nonunion or delayed union in our series.

There are several articles which are focusing on complications of treatment by TEN:

In January 2018 Rapp. M. and colleagues [19] published paper where they analysed results of treatment of femoral shaft fractures in overweight children (over 50 kg). From 12 patient (with insertion of two elastic nail) 7 patients had some shortening or instability of the fractures and they underwent second surgery. In the subgroup of 11 patients in whom third nail was inserted for more stability only one had poor outcome.

John M. Flynn et al. [11] analyzed 58 patients with treatment by TEN and showed that results were excellent or satisfactory in 57 of the 58 cases. In our study, we had almost the same amount of success - from 119 patients only 3 had poor outcomes. In another study [5] Flynn JM, Luedtke L, Ganley TJ, et al showed that from 50 cases the most common complication was irritation at the nail insertion site - 18 %. In our study 17 patient from total 119 (14%) had the same complaints.

Saikat Sarkar et al. [21] analyzed results of 70 patients and mentioned, that 14 patients out of 70 (20 %) had pain on the site of nail entry. For this reason 4 nails were removed. There was varus angulation in 4 cases out of 70 (5.71%), which did not require further treatment. They concluded that most of the complications were surgical technique related and pain at the nail entry site could be avoided by insertion of end cups. In our study not a single patient from 25 patients with insertion of end cups had any irritation or pain before performing the second surgery for removing the implants.

Windolf M. and colleagues [28] studied nail migration after end cups insertion in 15 artificial and 4 cadaveric femurs by creation and fixation of oblique pattern of fracture. They concluded that risk of elastic nail migration can be reduced by using of end cups.

Salonen A and et al. [22] in their study reported 9 postoperative complications out of 32 patients, which include nail prominence and instability. They concluded that short cutting of nail ends and proper diameter of nails can avoid these complications.

Unni G. Narayanan et al. [27] had reported 41 cases of pain or irritation by nail out of 77 patients; malunion in 8 cases, loss of reduction in 5 cases and refractures in 2 patients. They mentioned that most of the complications were minor and most are potentially avoidable. They recommended that nail ends be trimmed short and advanced so as to lie near metaphyseal cortices. During analysis of X-ray-s we mentioned, that almost all of

our patients, which had irritation and pain on nail insertion site, had preferably long ends above the entry point in the bone and we concluded, that if we cut nail ends preferably shorter or use end cups - we will resolve this complication.

Ernest L. Sink et al. [4] listed complications of TEN with different types of fractures. The complication rate for transverse fractures was 50% and 2 from 24 patients underwent surgery to resolve the complications. In group of unstable fractures (comminuted and long oblique fractures) rate of complications was 80% and 6 patients needed unplanned surgeries out of 15 patients. They concluded that for the long oblique and comminuted fractures it is better to consider other surgical techniques rather than TEN's. In our study we had 29 patient with type 2A oblique fractures (including long oblique fractures), but we had no major complications in this group.

In 2018 Reddy A and colleagues [20] analyzed 30 patients with close femoral shaft fractures treated with TEN. To assess the results they used Flynn criteria. 80% of patients whose weights were over 40 kg had satisfactory or poor results. Duration of surgery was 45 to 150 minutes and 57% of patients required open surgery. They had one major and 5 minor complication. They concluded that weight over the 40 kg is one of the factors, which can raise risk of complications. In our study we had 7 patients with weight between 40 to 50 kg and 4 of them had excellent results, 3 of them - satisfactory results (two patients about 10 degrees of varus deformation and one with irritation of nail end). 114 patients in our study had close reduction and only 5 patients out of 119 (0.04%) required open reduction.

Conclusion. Although there are some complications of TENs, listed in literature, with appropriate surgical technique, there are minimal and not important complications of these methods and majority of them can be avoided with right surgical techniques (For example by using of end cups for the nails) by experienced surgical team. This technique successfully can be used in heavy children (from 40 to 50 kg) and even in children more than 50 kg with fracture type A3. Stabilization by TEN is excellent method of treatment of first choice for children diaphyseal fractures.

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SUMMARY

COMPLICATIONS OF TREATMENT BY TITANIUM ELASTIC INTRAMEDULLARY NAILS IN CHILDREN WITH FEMORAL SHAFT FRACTURES

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Treatment of pediatric femoral shaft fractures is actual subject of modern traumatology, because of high frequency and different types of treatment. Nowadays Osteosynthesis by Titanium Elastic intramedullary nails (TEN) is the most popular method of treatment for pediatric shaft fractures. As an other methods of treatment Osteosynthesis by TEN also has some complications.

In our study we analyzed 119 patients 2 to 15 years of age with pediatric femoral shaft fractures who underwent operative treatment by TEN. According to Flynn's scoring criteria for TEN our patients had in 87 cases (73 %) excellent result, in 29 cases (24%) - satisfactory results and in 3 cases (3%) - poor results. For patients with satisfactory and poor outcome (total 32 patients) the main problem was different degree pain and irritation of soft tissues from nail ends. No one patients had the same problem in who was inserted nail "end cup" (total 25 patients). Average operation duration was 50 minutes (from 25 to 100) and in only 5 cases from 119 (4%) was needed open reduction for fracture. Average hospital stay for patients was 3 days (from 2 to 7 days). Patients were permitted begin partial weight bearing average for 5 week (range from 4 to 8 weeks). We had no one case of infection complication and non or delayed union.

We concluded that with operation by TEN the complications is mainly minor and don't affects general outcome of treatment for this particular kind of fracture. the majority of complications are surgery techniques related and can be avoided by using of end cups for the nails. This technique successfully can be used in heavy children (from 40 to 50 kg) and even in children more than 50 kg with fracture type A3. Osteosynthesis by Titanium elastic intramedullary nail is excellent method for treatment pediatric femoral diaphyseal fractures.

Keywords: pediatric femoral fracture; titanium elastic intramedullary nail.

РЕЗЮМЕ

ОСЛОЖНЕНИЯ ЛЕЧЕНИЯ ТИТАНОВЫМИ ЭЛАСТИЧНЫМИ ИНТРАМЕДУЛЛЯРНЫМИ СТЕРЖНЯМИ ПЕРЕЛОМОВ БЕДРЕННОЙ КОСТИ У ДЕТЕЙ

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Лечение переломов бедренной кости у детей по сей день является актуальной проблемой современной трав-

მათოლოგიაში მრავალრიცხოვანი შემთხვევების გამოკვლევის მიზნით ჩატარდა კვლევა, რომელიც ადასტურებს ტიტანის ინტრამედულარული სტერჯის (TEN) გამოყენების უსაფრთხოებას და ეფექტურობას მკურნალობის მეთოდის სახით.

სტატისტიკური მონაცემები აჩვენებს, რომ TEN-ის გამოყენების შემდეგ პაციენტების 73% (87) აღნიშნა კარგი შედეგი, 24% (29) - დაკმაყოფილებელი, ხოლო 3% (3) - არადაკმაყოფილებელი შედეგი. აღნიშნული შედეგები მიიღწეა მკურნალობის მეთოდის გამოყენების შედეგად, რაც ადასტურებს TEN-ის უსაფრთხოებას და ეფექტურობას მკურნალობის მეთოდის სახით.

შედეგების მიხედვით, TEN-ის გამოყენების შემდეგ პაციენტების 73% (87) აღნიშნა კარგი შედეგი, 24% (29) - დაკმაყოფილებელი, ხოლო 3% (3) - არადაკმაყოფილებელი შედეგი. აღნიშნული შედეგები მიიღწეა მკურნალობის მეთოდის გამოყენების შედეგად, რაც ადასტურებს TEN-ის უსაფრთხოებას და ეფექტურობას მკურნალობის მეთოდის სახით.

შეჯამება

ტიტანის ელასტიური ინტრამედულარული სტერჯის გამოყენების უსაფრთხოება და ეფექტურობა დასტურდა მკურნალობის მეთოდის სახით.

გ. ბაჯელიძე, ზ. ბერუაშვილი, ლ. ბაჯელიძე,
მ. ზიმლიცკი

დავით ტვილიძის სამედიცინო უნივერსიტეტი,
თბილისი, საქართველო

ბარძაყის დიაფიზის მოტეხილობის მკურნალობის მეთოდის სახით დასტურდა TEN-ის გამოყენების უსაფრთხოება და ეფექტურობა. აღნიშნული შედეგები მიიღწეა მკურნალობის მეთოდის გამოყენების შედეგად, რაც ადასტურებს TEN-ის უსაფრთხოებას და ეფექტურობას მკურნალობის მეთოდის სახით.

ტიტანის ელასტიური ინტრამედულარული სტერჯის (TEN) გამოყენების უსაფრთხოება და ეფექტურობა დასტურდა მკურნალობის მეთოდის სახით.

შედეგების მიხედვით, TEN-ის გამოყენების შემდეგ პაციენტების 73% (87) აღნიშნა კარგი შედეგი, 24% (29) - დაკმაყოფილებელი, ხოლო 3% (3) - არადაკმაყოფილებელი შედეგი. აღნიშნული შედეგები მიიღწეა მკურნალობის მეთოდის გამოყენების შედეგად, რაც ადასტურებს TEN-ის უსაფრთხოებას და ეფექტურობას მკურნალობის მეთოდის სახით.

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ON THE ISSUE OF NECESSITY TO PERFORM THE DR-70 IMMUNOASSAY PRIOR TO PROSTATE BIOPSY IN PATIENTS WITH HIGH PROSTATE SPECIFIC ANTIGEN LEVEL AND ITS EFFICACY IN PREDICTING THE BIOPSY RESULTS

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Prostate cancer (PCa) is the most seen cancer type on the men above 70 years of age [3]. One of the markers of PCa is serum prostate specific antigen (PSA) level. Although PSA level is organ specific, but it is not cancer specific. PCa is possible tumour in all PSA levels. Therefore, there is no consensus on PSA cutoff level yet. The possibility of high PSA levels due to many causes (infection, catheterization, digital rectal examination etc.) outside of cancer makes it difficult to perform a good cancer screening. 4 ng / ml, which is considered to be a threshold value of PSA, has been replaced by 2.5 ng/mL for the last few years [18]. But Ross et al. have reported that a cut-off of 1.5 ng/mL should be used in the decision of prostate biopsy. So management of borderline PSA is not straightforward [20]. For patients with high PSA levels or patients that have a significant finding on digital rectal examination (DRE), a transrectal ultrasonography-guided prostate biopsy should be applied according to National Comprehensive Cancer Network (NCCN) and European Association of Urology (EAU) guidelines [6,17].

The morbidity and high cost associated with TRUS guided prostate biopsy is great importance to both patients and clinicians alike [12,16]. New biological markers such as Prognostic PCA3 and SelectMDX DRE urine tests, the serum 4Kscore and Prostate Health Index (PHI) have been evaluated due to insufficient PSA level in prostate biopsy decision. It is clear that these tests shown to add sensitivity and specificity on top of PSA, potentially avoiding unnecessary biopsies or lowering over-diagnosis and are reduced the high cost of biopsy in health management [4,26,27].

An association between malignancy and the coagulation system was reported by Trousseau more than a century ago [25]. Fibrin and fibrin degradation products (FDP) have been investigated for many years in oncological researches. In malignancy tissue, fibrin is a component of the connective tissue stroma. But fibrinogen is more widely located in the connective tissue [9]. The studies in the literature that support the increase of hemostatic abnormalities in cancer cases. Overt or subclinical intravascular coagulation usually occurs in patients with malignancy [19] and this is closely related to the fibrinolysis process. The increased FDP level due to fibrinolysis in patients with malignancy is the most common hematological abnormality [23]. These FDP products are important due to the fibrinogen act as pathogenetic connecting links for development and spread of malignant tumors on the initial phase of haematogenic metastasis particularly [14].

Polyclonal antibody DR-70 as a tumor marker is an ELISA-based laboratory test with high specificity and sensitivity to measure fibrin and FDP in the blood, even in the early stages of cancer. American FDA and European CE is an approved DR-70 immunoassay. It is not an organ-specific marker. DR-70 levels were found to be increased in 13 different tumors [28]. Unlike other tests, DR-70 immunoassay evaluates all fibrin and FDPs including X, Y, D, E, D-dimer particles.

In studies on the DR-70 immunoassay in genitourinary tumors, results related to bladder and prostate cancer have been published [21,24,28]. However, there is no information using of

this test prior to prostate biopsy in these studies. Therefore, its contribution to PSA is not clear.

In this study, we aimed to discuss the contribution of the DR-70 for the patients with high PSA level and which cutoff level of DR-70 must be taken into account the biopsy decision.

Material and methods. 453 cases were performed transrectal ultrasonography (TRUS) guided prostate biopsy due to borderline or high PSA level or significant DRE findings in our clinic between January 2016 and March 2018 were evaluated. 93 cases whose data were reached taken into the study.

The inclusion criteria of the patients were; To have applied to the general surgery department within the last 3 months and to be measured as a DR-70 level screening test, to have a borderline or high PSA level. Patients with diagnosed a disease of coagulopathies or urinary tract infections, patients who have had surgery in the past year or patients who were used anti-androgen and 5-alfa reductase inhibitory treatment and patients with inadequate data were also excluded from the study.

Factors that could increase the level of DR-70 were evaluated. Patients with a known malignancy were not included in the study. All patients were questioned for possible colorectal cancer. The patient who had constipation, rectal bleeding, etc. was not included in the study.

The study was designed as a prospective observational cohort study. The study was conducted among 56 patients with benign (Group 1 or G1) and 37 patients of malignant (Group 2 or G2) biopsy result. The patient's medical records were recorded. Patients age, grading and findings of digital rectal examination, prostate volume (PV) which was calculated with the ellipsoidal method (length X depth X width X π X 1/6) by TRUS, total PSA (tPSA) and free PSA level (fPSA), rate of percentage of free to total prostate specific antigen (f/tPSA), PSA density (PSAD) which was analysed as total PSA (ng/mL) divided by prostate volume (ml) and DR-70 level, number of received cores and pathology results were recorded for both of biopsy.

The fibrin degradation products were quantitatively evaluated using DR-70 kits (AMDL, Inc., Tustin, CA, USA). The blood samples were stored in siliconized vacutainers with gel serum separators (SST vacutainer). Centrifugation of the samples was performed for 30 min–4 h after venipuncture [28]. All samples were kept frozen at -80°C until assay. During the immunoassay, the immobilized rabbit anti-DR-70 (FDP) polyclonal antibodies and horseradish peroxidase-conjugated polyclonal anti-human fibrinogen antibodies were used. The serum levels of FDP were determined by interpolating the absorbance at 450 nm from the provided standard curve [2].

One day before the procedure, oral administration of 500-mg levofloxacin and 400-mg etodolac was started and it was continued after the biopsy. The day of biopsy a rectal enema (250 mL) was performed before the biopsy. The biopsy was performed while the patient was in the left lateral position with the thighs flexed. The procedure was performed under the guidance of ultrasound device with a 7.5 MHz biplanar probe.

The biopsy was performed on an outpatient basis in a room equipped with all material necessary for emergency interven-

tion. Sedation and anesthesia were not achieved. 10 minutes before the procedure, periprostatic nerve blockade was performed in addition to perianal intrarectal lidocain gel. Injections were delivered at the angle between the seminal vesicle and prostate on each side using 5 cc of 2% lidocain. The biopsies were performed by multiple experienced urologists. In initial biopsy, standard 12 (both lateral and medial biopsies from the base, medial and apex on the right and left side of the prostatic peripheral zone) core prostate biopsy was performed.

Statistical analyses were performed using SPSS Statistics 22.0 software (SPSS Inc., Chicago, IL, USA) and Microsoft excel computer programs. The normality hypothesis was tested using the Kolmogorov-Smirnov test during data analysis. Descriptive levels are presented as medians and minimum/maximum for other than normally distributed. Mann-Whitney U test was applied for mean difference comparison between G1 and G2. Chi-Square was used to assessment difference comparison between gleason score of PCa and DR-70 level. Spearman's rho was used to analyze the linear relationship between DR-70 level and gleason score of PCa. Receiver operating characteristic (ROC) curves were used to quantify the predictive accuracy of the logistic models. The diagnostic significances of tPSA, fPSA, f/tPSA, PSAD and DR-70 levels on biopsy result was evaluated by ROC curve. We constructed ROC curves by plotting sensitivity against 1 - specificity. Two-tailed levels of $P < .05$ were considered statistically significant.

Results and their discussion. The median age of patients in G1 and G2 was 62.52 and 68.22 years, respectively. The mean PV in G1 and G2 were 52.16 and 39.6 mL, respectively. The mean tPSA, fPSA, f/tPSA rate, PSAD and DR-70 levels in G1 and G2 were found as 7.19 and 18.74 ng/mL, 1.6 and 3.84 ng/mL, 0.21 and 0.18, 0.14 and 0.48 ng/mL/cc and 0.44 and 0.5 µg/mL, respectively (Table). The mean DR-70 levels according to gleason score of PCa (3+3, 3+4, 4+3, 4+5 and 5+5) were 0.42, 0.59, 0.71, 0.43 and 1.43 µg/mL, respectively.

The mean age of the patients in G2 was statistically significantly higher than G1 ($p = .001$). The mean PV of the patients in G2 was statistically significantly lower than G1 ($p = .001$). There was no statistically significant difference on tPSA, fPSA and f/tPSA levels between G1 and G2 ($p = .12$, $p = .32$ and $p = .10$, respectively). The mean PSAD of the patients in G2 was statistically significantly higher than G1 ($p = .001$). There was no statistically significant difference on DR-70 levels between G1 and G2 ($p = .38$) and on DR-70 levels according to gleason score of PCa in G2 ($p = .51$). In Spearman's rho correlation analysis,

there was no statistically significant relationships between DR-70 levels and pathology results in G2 ($p = .24$). ROC curve of tPSA, fPSA, f/tPSA, PSAD and DR-70 levels were evaluated. ROC curve of PSAD shows a fair discriminant power with $AUC = 0.71$ (95% CI: 0.607–0.828) for differentiation between PCa and benign tissue in prostate biopsy with moderate specificity and high sensitivity (62.5% and 75.7%, resp., cut-off level: 0.1377 ng/mL) (Fig.).

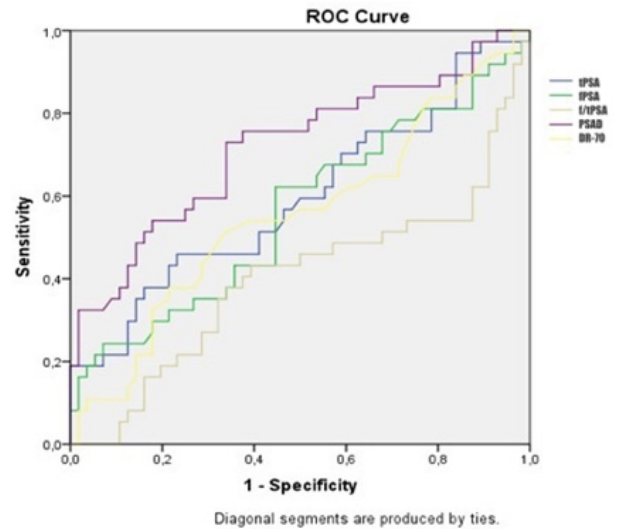


Fig. ROC curve of PSAD shows a fair discriminant power with $AUC = 0.71$ (95% CI: 0.607–0.828) for differentiation between PCa and benign tissue in prostate biopsy with moderate specificity and high sensitivity (62.5% and 75.7%, resp., cut-off level: 0.1377 ng/mL)

Prostate cancer is the second most commonly diagnosed cancer in men [13]. One of the most important indicators at diagnosis is serum PSA level. Besides high PSA level, findings such as nodule existence in prostate tissue during DRE, asymmetry, and palpable induration of the prostate affect biopsy decision. While cut-off level of PSA level is contradictive, the leading factor of prostate biopsy decision.

In our study, The mean age of the patients in G2 was higher than G1, and it complies with literature. The main parameter for deciding prostate biopsy is tPSA and trustful used in daily practice. PSA has an admissible sensitivity for prostate cancer

Table. The results of parameters performed before prostate biopsy and the statistical evaluation of the measurements between in two groups

Variables	Group 1 Median (Min.-Max.)	Group 2 Median (Min.-Max.)	p value
Age (years)	62.52 (50-78)	68.22 (56-80)	.001*
Prostate Volume (ml)	52.16 (20-90)	39.6 (10-120)	.001*
tPSA(ng/mL)	7.19 (1.04-24.5)	18.74 (0.81-100)	.12
fPSA(ng/mL)	1.6 (0.15-12.6)	3.84 (0.01-30)	.32
f/tPSA	0.21 (0.04-0.5)	0.18 (0.01-0.33)	.10
PSAD (ng/mL/cc)	0.14 (0.02-0.46)	0.48 (0.04-2.78)	.001*
DR-70 (µg/mL)	0.44 (0.13-2.01)	0.48 (0.17-1.74)	.38

*: $p < .05$

tPSA - Total Prostate-Specific Antigen, fPSA - Free Prostate-Specific Antigen, f/tPSA - Free/Total Prostate-Specific Antigen, PSAD - Prostate-Specific Antigen Density, DR-70: an ELISA based kit for evaluating of fibrin degradation products

with 4 ng/mL cut-off level [7]. Catalona et al. was reported that it was stated that PSA cut-off level must be 2,5ng/mL, especially for young patients [8]. In another study Arcangeli et al. found that PCa can be missed approximately at the rate of 20-30% when PSA cut-off level 4 ng/mL was used [1]. In our study, the cut-off level of tPSA was preferred as 2.5 ng/mL. Only 2 (5.4%) of the 37 patients with PCa had a tPSA level under 2.5 ng/mL in G2. This is parallel to discussions on cut-off levels issue, and it shows prostate cancer diagnosis can be missed on low tPSA levels. Being between 4-10 ng/mL of PSA level is called grey zone. Because high level of negative results gained on biopsies on the patients with grey zone PSA levels, in the course of time new parameters such as fPSA, f/tPSA rate, PSAD and PSA velocity are started to be evaluated. Braver et al. was reported that patients in the grey zone can be diagnosed prostate cancer with 95% rate when the f/tPSA threshold level is considered 25%. Thus, 20% of the patients can be saved from the unnecessary biopsy [5]. Seaman et al. and Kotb et al. were reported if PSAD threshold level is taken as 0,15ng/mL/cc, there can be an improvement in detecting prostate cancer [15,22]. In our study, the mean tPSA level was 7.19 and 18.74 ng/mL ng/mL in G1 and G2, respectively and conformed the grey zone in G1. In this zone, tPSA level is not enough to evaluate for patients, parameters such as fPSA, f/tPSA rate and PSAD should be examined. Detecting f/tPSA rate as 0.21 in G1, considering Braver et al.'s study, it supports our results' accuracy and reliability. Also in our study, PSAD was 0.14 and 0.48 ng/mL/cc in G1 and G2, respectively and when threshold level was considered as 0.15 ng/mL/cc, this outcome in benign patients support the importance of evaluating PSAD. This result support the Seaman and Kobt et al.'s study [15,22] regarding PSAD. Biological markers such as tPSA, fPSA, f/tPSA and PSAD are valuable for prediction of PCa. However, in our study, the most valuable marker was PSAD. As the cutoff level 0.13 ng/mL/cc can be used in benign / malignant tissue differentiation. Evaluation of PSAD as a support to tPSA will usually give enough results. As we reported in our study, we recommend the use of PSAD and tPSA together without any further investigation.

Prostate volume is another effective factor in predicting of PCa. Erdogan et al. have reported that PV was a significantly better indicator of PCa than PSAD and f/t PSA [11]. Demura et al. have shown that PV is associated with a decrease in size and detectability of cancer lesions and 50 mL is a cutoff for that [10]. In our study, the mean PV was 39.6 mL and lower volumes of prostate tissue is more associated with PCa than higher PVs. In contrast to the study by Erdogan et al., in our study, PSAD was found to be a more meaningful parameter in the diagnosis of PCa. We recommend that the biopsy decision should be made more easily in patients with low PV (<50 mL).

Fibrin and fibrin degradation products are molecules that have proven effective in cancer tissue. Cutoff level of DR-70 in healthy population is 0.5 µg/mL (0.2-0.8) [29]. In our study, DR-70 levels in both groups were 0.44 and 0.5 µg/mL, respectively, and were not different from the normal population. The DR-70 level was found to be higher in G2, even if it was not statistically significant. However, its effectiveness in the urinary system is weaker than other systems. Unfortunately, there is no contribution in the diagnosis of PCa. It can not distinguish PCa tissue from normal tissue and does not correlate with cellular grade increase in PCa tissue. Therefore, there is no need for additional evaluation before prostate biopsy. Considering that PCa is more common after 70 years of age, it can give an idea of another concurrent malignancy.

The most successful parameter for prostate cancer prediction was determined as tPSA and PSAD. We recommend that PSAD be considered in the biopsy decision. The threshold value of 0.13 has high sensitivity and specificity for biopsy decision. The DR-70 does not contribute to this decision, and its use in prostate cancer has no clinically significant benefit.

This study contains several limitations. The low number of patients in the cohort and the DR-70 level not being evaluated simultaneously with the prostate biopsy are the limitations of the study.

Conclusions. Before the prostate biopsy, tPSA, fPSA and f/tPSA rate should be routinely evaluated in the patients with suspicion of PCa. Contrary to literature and guidelines, cutoff level of PSAD as 0.13 ng/mL/cc should be kept in mind and accordingly, a biopsy decision should be made. We think that DR-70 is no needed for additional evaluation before prostate biopsy. In the future, we think that the DR-70 level should be investigated with larger patient series molecularly in prostate tissue.

EC contributed to the conception and design of the study, EC and AS collection of data, TCM and THH drafting, EC revision the manuscript, OY and KO in preparing tables and performing statistical analysis. Author read and approved the final manuscript.

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SUMMARY

ON THE ISSUE OF NECESSITY TO PERFORM THE DR-70 IMMUNOASSAY PRIOR TO PROSTATE BIOPSY IN PATIENTS WITH HIGH PROSTATE SPECIFIC ANTIGEN LEVEL AND ITS EFFICACY IN PREDICTING THE BIOPSY RESULTS

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The aim is to discuss the contribution of the DR-70 for the patients with high PSA level and which cutofflevel of DR-70 must be consideredthe biopsy decision.

93 patients with high prostate specific antigen level were enrolled into the study. Before the prostate biopsy, total PSA (tPSA), free PSA (fPSA), free/total PSA rate (f/tPSA), PSA density (PSAD) and DR-70 levels were recorded. The patients were divided into two groups according to the pathological outcome of benign (G1) or malignant (G2). G1 and G2 were compared with Mann-Whitney U test, Spearman's rho and ROC curve for analysis. The significance level is taken as .05 for all tests.

The median age of patients in G1 and G2 was 62.52 and 68.22 years, respectively. The mean PV in G1 and G2 were 52.16 and 39.6 mL, respectively. The mean tPSA, PSAD and DR-70 levels in G1 and G2 were found as 7.19 and 18.74 ng/mL, 0.14 and 0.48 ng/mL/cc and 0.44 and 0.5 µg/mL, respectively.

The mean age of the patients in G2 was statistically significantly higher than G1 (p=.001).The mean PV of the patients in G2 was statistically significantly lower than G1 (p=.001).The mean PSAD of the patients in G2 was statistically significantly higher than G1 (p=.001). There was no statistically significant difference on DR-70 levelsbetween G1 and G2 (p=.38). In Spearman's rhocorrelationanalysis, there was nostatistically significant relationships between DR-70 levels and pathology results in G2 (p=.24). ROC curve of tPSA, fPSA, f/tPSA, PSAD and DR-70 levelswere evaluated. ROC curve of PSAD shows a fair discriminant power with AUC = 0.71 (95% CI: 0.607-0.828) for differentiation between PCa and benign tissue in prostate biopsy with moderate specificity and high sensitivity (62.5% and 75.7%, resp., cut-off level: 0.1377 ng/mL).

Contrary to literature and guidelines, cutoff level of PSAD as 0.13ng/mL/cc should be kept in mind and accordingly, a biopsy decision should be made. We think that DR-70 is not needed for additional evaluation before prostate biopsy.

Keywords: DR-70, PSA, prostate biopsy, prostate cancer, PSA density.

РЕЗЮМЕ

ИММУНОФЕРМЕНТНЫЙ АНАЛИЗ DR-70 И БИОПСИЯ ПРЕДСТАТЕЛЬНОЙ ЖЕЛЕЗЫ: НЕОБХОДИМОСТЬ ИММУНОФЕРМЕНТНОГО АНАЛИЗА DR-70 ПЕРЕД БИОПСИЕЙ ПРЕДСТАТЕЛЬНОЙ ЖЕЛЕЗЫ У ПАЦИЕНТОВ С ВЫСОКИМ УРОВНЕМ ПРОСТАТИЧЕСКОГО СПЕЦИФИЧЕСКОГО АНТИГЕНА И ЕГО ЭФФЕКТИВНОСТИ В ПРОГНОЗИРОВАНИИ РЕЗУЛЬТАТА БИОПСИИ

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Цель исследования - определить эффективность иммуноферментного анализа DR-70 для мониторинга пациентов с высоким уровнем простатспецифического антигена.

В исследование включены 93 пациента с высоким уровнем простатспецифического антигена (PSA). Пациенты в зависимости от характера опухоли разделены на две группы: доброкачественная (G1 - первая группа - 56 пациентов) и злокачественная (G2 - вторая группа - 37 пациентов). Средний возраст пациентов в группах G1 и G2 составил 62,52 и 68,22 года, соответственно и статистически значимо был выше в группе G2 ($p=.001$). Перед биопсией простаты определены общий PSA (tPSA), свободный уровень PSA (fPSA), уровень свободного/общего PSA (f/tPSA), плотность PSA (PSAD), объем простаты (PV) и уровень DR-70. Показатели пациентов групп G1 и G2 сравнивались по следующим критериям: U-критерий Манна-Уитни, коэффициент ранговой корреляции Спирмена и ROC-кривая.

Исследованы факторы, повышающие уровень DR-70. Средние значения PV в G1 и G2 составили 52,16 и 39,6 mL, соответственно. Средние уровни tPSA, PSAD и DR-70 составили 7,19 и 18,74 ng/mL, 0,14 и 0,48 ng/mL/cc и 0,44 и 0,5 $\mu\text{g/mL}$, соответственно. Средняя PV пациентов в группе G2 была статистически значимо ниже, чем в группе G1 ($p=.001$); средняя PSAD статистически значимо выше ($p=.001$). Статистически значимой разницы в уровнях DR-70 в G1 и G2 не выявлено ($p=.38$). Корреляционный анализ Спирмена статистически значимых взаимосвязей между уровнем DR-70 и патологией в группе G2 ($p=.24$) не выявил. Изучены ROC кривая уровней tPSA, fPSA, f/tPSA, PSAD и DR-70. В результате проведенного исследования, в противоположность данным научной литературы, установ-

лено, что биопсию следует проводить при минимальном уровне PSAD 0.13ng/mL/cc и нет необходимости определять показатели DR-70.

რეზიუმე

DR-70-ის იმუნოფერმენტული ანალიზი და წინამდებარე ჯირკვლის ბიოფსია: DR-70 იმუნოფერმენტული ანალიზის აუცილებლობა წინამდებარე ჯირკვლის ბიოფსიის წინ პაციენტებში პროსტატის სპეციფიკური ანტიგენის მაღალი დონით და მისი ეფექტურობა ბიოფსიის შედეგის პროგნოზირებაში

ჯ.ედიზი, ს.აკანი, ჯ.ტემელი, ხ.ტავუკჯუ, ო.ილმაზი

სულთან აბდულხამიდის სახ. საგანმანათლებლო და კვლევითი ჰოსპიტალი, სტამბული, თურქეთი

კვლევის მიზანს წარმოადგენდა DR-70-ის იმუნოფერმენტული ანალიზის ეფექტურობის განსაზღვრა პროსტატის სპეციფიკური ანტიგენის (PSA) მაღალი დონის პაციენტების მონიტორინგისათვის.

კვლევაში ჩართული იყო 93 პაციენტი PSA-ს მაღალი დონით. პროსტატის ბიოფსიის წინ განისაზღვრა PSA-ს საერთო დონე (tPSA), თავისუფალი დონე (fPSA), PSA-ს სიმკვრივე (PSAD), პროსტატის მოცულობა (PV) და DR-70 დონე.

სიმსივნური პროცესის ტიპის გათვალისწინებით პაციენტები გაყოფილი იყო 2 ჯგუფად: კეთილთვისებიანი (G1 - პირველი ჯგუფი, n=56) და ავთვისებიანი (G2 - მეორე ჯგუფი, n=37). პაციენტების საშუალო ასაკი ორივე საკვლევ ჯგუფში შეადგენდა 62,52 და 68,22 წლ., შესაბამისად, და სტატისტიკურად სარწმუნოდ უფრო მაღალი იყო G2 ჯგუფში ($p=.001$). გამოკვლეული იყო ფაქტორები, რომლებიც ზემოქმედებენ DR-70 დონეზე. PV საშუალო მაჩვენებელი G1 და G2 ჯგუფებში შეადგენდა 52,16 და 39,6 მლ, შესაბამისად; tPSA, PSAD და DR-70 საშუალო დონე - 7,19 და 18,74 ng/mL, 0,14 და 0,48 ng/mL/cc, 0,44 და 0,5 $\mu\text{g/mL}$, შესაბამისად. PV-ს საშუალო მაჩვენებელი G2 ჯგუფში სტატისტიკურად სარწმუნოდ დაბალი იყო G1 შედარებით ($p=.001$). საშუალო PSAD კი, პირიქით, სარწმუნოდ უფრო მაღალი ($p=.001$). სტატისტიკურად სარწმუნოდ განსხვავება DR-70 მაჩვენებლებს შორის არც ერთ ჯგუფში არ გამოვლინდა ($p=.38$). სპირმენის კორელაციის ანალიზმა G2 ჯგუფში DR-70-ის დონესა და პათოლოგიას შორის სტატისტიკურად სარწმუნოდ კავშირი არ გამოავლინა ($p=.24$). შესწავლილია ROC-მრუდები tPSA, fPSA, f/tPSA, PSAD და DR-70.

სამეცნიერო ლიტერატურაში მოყვანილი მინაცემების საპირისპიროდ, ჩატარებული კვლევის შედეგად დადგინდა, რომ ბიოფსიის ჩატარება საჭიროა PSAD-ის მინიმალურ მაჩვენებლის შემთხვევაში (0.13ng/mL/cc), ხოლო DR-70 განსაზღვრა არ წარმოადგენს აუცილებლობას.

DYNAMICS OF FUNCTIONAL CAPABILITIES AMONG 17-22 YEARS OLD GIRLS WITH DIFFERENT VEGETATIVE STATUS DURING THE OVARIAN-MENSTRUAL CYCLE

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The condition of organism of girls throughout their reproductive years is constantly influenced by the dynamic fluctuations of their hormonal background. The effect from influence of cyclic changes of hormonal secretion on all organ systems has been studied for a long time [1]. As a rule, researchers are interested in adaptation changes in the organism of girls engaged in a certain sport or having a high level of sports qualification, but data on the functional state of girls with low motor activity are practically non-existent. It must be noted, that today there is still ambiguity in scientific data regarding the nature of changes in women's functional capabilities over the menstrual cycle [5,6,8,11,15–17], in particular, the adaptation reactions of the cardiovascular system (CVS) [2,3,14,18,23].

The study of the dynamics of the mechanisms of regulation of cardiac activity of girls with low motor activity (LMA) during the ovarian-menstrual cycle (OMC) has a certain scientific-theoretical and practical interest. On the one hand, the state of CVS can be considered as an indicator of the reserves of functional capabilities, because it is the most sensitive to a variety of different factors [1, 2], and the level of activity and the adequacy of CVS reactions, in particular, to physical activity, is determined by the state of control mechanisms of the autonomic nervous system, which is described by changes in the heart rate [2,3,14,22,25]. On the other hand, girls 17–22 years old with LMA are under the influence of more stable endocrine fluctuations compared to sportswomen.

Objective – to study the dynamics of functional capabilities of girls of 17–22 years old with different vegetative status during the ovarian-menstrual cycle.

Material and methods. 28 girls took part in research – there were students of Faculty of Primary Education, Preschool Pedagogy and Psychology, whose physical activity was limited to one physical activity per week. Among many classifications of the menstrual cycle (MC) phases the most optimal is the classification proposed by the Institute of Gerontology AMS of Ukraine, which consists in dividing the menstrual cycle into 5 phases: menstrual (I), postmenstrual (II), ovulatory (III), postovulatory (IV) and premenstrual (V), according to which the study was conducted. The menstrual cycle of girls was not violated for 3 months, which made it possible to use the calendar method of phase determination by N.V. Svechnikova [5,9,17].

All research was conducted on the basis of the Department of Biology and Health Protection at the Laboratory of Age Physiology of Sports of South Ukrainian National Pedagogical university named after K.D. Ushinsky (Odessa) with observance of the basic bioethical provisions of the Convention of the Council of Europe on Human Rights and Biomedicine (04.04.1997), the Declaration of Helsinki of the World Medical Association on ethical principles of scientific medical research with the participation of human beings (1964-2008), as well as the Order of the Ministry of Health of Ukraine № 690 of 23.09.2009.

The following methods were used to achieve this goal: questioning, anthropo-physiometry, electrocardiography with the following definition of the variability of the heart rate by R.M. Baevsky [2,3], cycle ergometry, the calendar method for determining the phases of the menstrual cycle, blood pressure mea-

surement of systolic, diastolic, pulse (ADs, ADD, PD), calculation of systolic and minute blood volume, statistical methods for processing research results.

Work on the cycle ergometer VED-12 was used as a muscle load according to D.N. Davidenko's methodology [7], modernized in our laboratory. Testing consisted in a smooth, continuous increase in load power from zero to heart rate (HFR) 150–155 beats per minute, after which, according to the program the operating power was reduced to the initial level with the same speed.

Heart rate variability (HRV) was studied before cycle ergometric loading (at rest), at the moment of reverse (at HFR=150–155 beats per minute), at the end of work and at the 5th minute of recovery, sitting on the cycle ergometer. To assess HRV, a series of indicators characterizing the state of the autonomic nervous system was determined: Mo (c), AMo (%), ΔX (c). Based on these indicators, the indices were calculated on the proposed R. M. Baevsky [2], which are used to assess the regulation and adaptation of the cardiovascular system to physical activity – activity of the humoral regulation channel (AHRC, c. u.), vegetative rhythm indicator (VRI, c. u.), vegetative equilibrium index (VEI, c. u.), indicator of the adequacy of regulatory processes (IARP, c. u.) and regulatory voltage index (RVI, c. u.).

Statistical processing of the results was carried out using the SPSS 16 application software. The obtained results are presented in the form of $M \pm m$, where M is average and m is average error. To compare the dynamics of the indicators were used to determine the Wilcoxon criterion and the Mann-Whitney U-test. Reliable differences were considered to be differences at $p < 0.05$.

The work was done in accordance with the plan of research work of the Department of Biology and Health Protection of University named after K.D. Ushinsky «Systemic adaptation to physical and mental stress at certain stages of human ontogenesis» (N state registration. 0109U000206), «Adaptation of children and young people to educational and physical activities (boys aged 17–21)». (N state registration 0114U007158).

Results and their discussion. The physical development of the surveyed girls according to the mean group anthropo-physiometric data was in compliance with age and sex standards [5]. It is common knowledge, that women of reproductive age change the activity of the vegetative nervous system and the humoral regulatory channel during the menstrual cycle under the influence of estrogen and progesterin concentration (gestogens) accordingly, parasympathetic and cholinergic influences predominate in the first half of the cycle, and sympathetic and adrenergic influences predominate in the second half [10,17,19,20,24]. In the state of relatively rested muscles, in each phase of MC, we determined the state of vegetative type of regulation (vagotony, normotonia, sympathicotonia) on the level of stress index. Other criterias, such as those integrated into the RVI calculation, had corresponding changes in RVI dynamics of fluctuations.

The first – vagotonic - type of regulation was observed in 35.7–60.7% of girls. It was mostly manifested in postmenstrual (60.7%) and postovulatory (53.6%), the lowest – in premenstrual (39.3%) and menstrual (35.7%) phases of MC. Eutonic type of influence included 7.1–35.7 % of the surveyed persons. This type of regulation was observed in a higher number of girls

in the premenstrual (35.7%), menstrual (32.2%) and ovulatory (32.2%) phases. The third type of regulation, sympathicotonic, indicated a reduction of adaptation possibilities in 21.4% of girls in the postmenstrual and ovulatory phases and in 32.1% in the menstrual phase, and 39.3% in the postovulatory phase of OMC.

To assess the peculiarities of vegetative regulation of the cardiovascular system of girls and its reactions to muscular activity were tested in a closed cycle (with reversal) with dosed physical activity. The results of ergometry showed unreliable ($p > 0.05$) higher efficiency, according to PWC_{170} , in postmenstrual (129.62 ± 3.58 W) and premenstrual (133.52 ± 3.37 W), and a little lower ($p > 0.05$) – in ovulatory (127.08 ± 4.17 W) and post-ovulatory (127.34 ± 3.18 W) phases of MC, which can demonstrate the limiting role of menstruation and ovulation processes in the manifestation of functional capabilities [13,17]. However, the data obtained do not allow to fully assessing the degree of influence of vegetative regulation on the mechanisms of adaptation to physical activity in different phases of OMC. To solve this problem, we have divided the general group into subgroups in accordance with the type of heart rate regulation defined in the menstrual phase of MC.

As the results of individual analysis of initial HRV data have shown, the girls with predominance of the vagotonic type of vegetative regulation showed higher physical performance on the background of low estrogen-progesterone concentration in menstrual (I) and premenstrual (V) and lower - on estrogen peaks - in ovulatory (III) and postovulatory (IV) phases of OMC (Fig. 1). It is unreliable ($p > 0.05$) that the higher physical performance of girls in phases I and V was followed by a significant tension of regulatory mechanisms, which is confirmed by the higher tension of regulatory mechanisms (RVI) during the whole testing in comparison with other phases of MC.

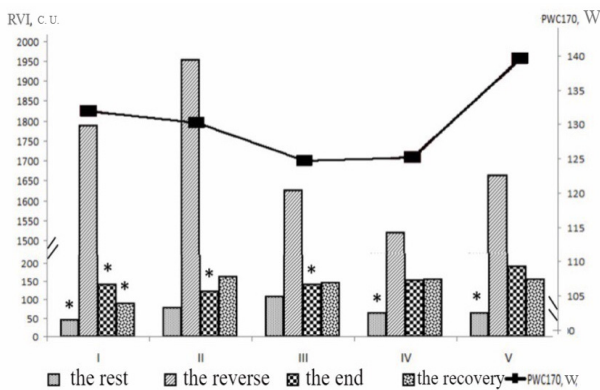


Fig. 1. Dynamics RVI and PWC_{170} girls 17–22 years old with vagotonic type of heart rate regulation during the ovarian-menstrual cycle (I–V phases of OMC) during closed cycle testing (with reverse). Note: * – there are significant differences between vagotonic and normotonic girls ($p < 0.05$)

It must be noted that in the menstrual phase the recovery processes were more intensive than in the premenstrual phase of MC and were characterized by the increased influence on the cardiac activity of the parasympathetic part of the autonomous nervous system, which indicates the resistance of girls of 17–22 years old with a vagotonic type of heart rate regulation to physical activity and a higher potential reserve (increase of RVI values by 50.1% and 59.1%, correspondingly, in phase I and V). The low performance of the vagotonics in phases III and IV was

determined, on the one hand, by a moderate level of regulation voltage before testing and on the other hand, by the high reactive mobilization of adaptive reserves in the process of muscular work. At the same time, adaptation to the stress varying in a closed cycle was accompanied by more costly physiological mechanisms due to the activation of sympatho-adrenal system, which is indicated by the fixed values of cardiac rhythm at rest and at the end of the stress (increase in the values of RVI by 23.2% and 58.3%, respectively, in phases III and IV).

The girls with normotonic type of ANS have performed a larger volume of work and accordingly, have demonstrated high values of PWC_{170} , in menstrual (133.47 ± 3.68 W) and ovulatory (135.2 ± 8.4 W) and smaller – in postmenstrual (129.22 ± 7.44 W) and postovulatory (126.45 ± 7.18 W) phases of MC (Fig. 2) that testifies to significant adaptation reserves in the physiological stress phases, in spite of the established opinion [5,12,17,21].

The higher work capacity of normotonics in the menstrual phase, as compared to vagotonics, was accompanied by a higher tension of regulatory mechanisms (RVI) at rest (111.93 ± 5.84 c. u.), on the reverse of the load (1551.12 ± 180.3 c. u.) and a low intensity of regenerative processes (196.62 ± 35.24 c. u.). The initial state of HRV of girls-normotonics in the ovulatory phase corresponded to the middle of the age norm (89.18 ± 11.36 c. u.) and was characterized by a significant, but smaller reaction to the load in relation to the shifts in the menstrual phase (RVI on the reverse increased to 1088.2 c. u.). By the time of leaving the load RVI decreased to 883.95 c. u. and significantly decreased after 5 minutes of rest after work (to 94.8 c. u.), which indicates a more optimal level of functional capacity mobilization in this phase for the manifestation of general physical performance and preservation of homeostasis.

The decreased adaptive capacity, and consequently the PWC_{170} , in the postmenstrual (129.22 ± 7.44 W) and postovulatory (126.45 ± 7.18 W) phases was accompanied by a high reactive response (RVI 1838.08 ± 196.41 c. u. and 1541.42 ± 177.21 c. u., correspondingly) and slower processes of restitution (150.85 ± 26.34 c. u. and 185.4 ± 22.06 c. u., correspondingly).

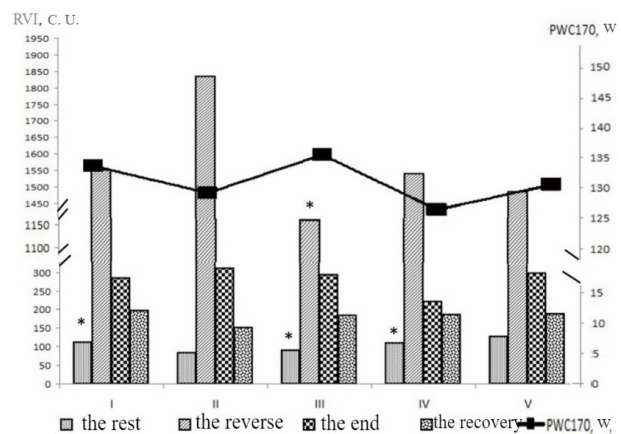


Fig. 2. The dynamics of RVI and PWC_{170} girls of 17–22 years old with normotonic type of heart rate regulation during the ovarian-menstrual cycle (I–V phase of OMC) during the closed cycle testing (with reverse). Note: * – the significant differences between the data of normotonic and sympathicotonic girls ($p < 0.05$)

The examined persons with ANS sympathicotonic type in contrast to normotonics carried out muscle loading with better results in postmenstrual (129.39 ± 5.69 W) and postovulatory (130.95 ± 7.27

W) phases and with the worst results in menstrual (119.17 ± 8.14 W) and ovulatory (121.94 ± 6.48 W) phases of MC (Fig. 3). The same results were obtained by other researchers, who indicate a natural decrease in stress in these phases [5,11,13,17].

In fact, as in the postmenstrual (121.96 ± 26.48 c. u.), as in the postovulatorial (67.7 ± 11.5 c. u.) phases, the level of regulation mechanisms tension at rest was reliably ($p < 0.05$) lower, and its increase in the process of cycle ergometric test was characterized by a smaller diapason in comparison with other phases (RVI in these phases was, respectively, on the reverse and at the end of the load 1334.88 c. u. and 1223.25 c. u.; 1105.88 c. u. and 1085.86 c. u.). The recovery was also faster than in other phases of the menstrual cycle.

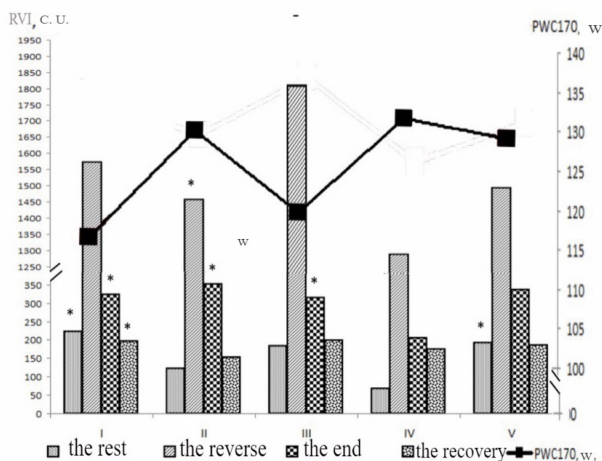


Fig. 3. The dynamic of RVI and PWC_{170} of girls 17–22 years old with sympathicotonic type of cardiac rhythm regulation during the ovarian-menstrual cycle (I–V phases of CMCs) during closed cycle testing (with reverse). Note: * – there are significant differences between the data of sympathicotonic girls and vagotonics ($p < 0.05$)

Significant level of activation of heart rate regulation mechanisms in relative rest in menstrual, ovulatory and premenstrual phases, according to the criteria of R. M. Baevsky, indicates a significant strain on regulatory systems, which was more likely to be detected and maintained throughout testing when girls were exposed to physical activity. It is notable that during these phases a part of the studied (44.4–66.7%) had lower IN by 5.68–25.88 c. u. during the recovery period than at rest, which indicates the imbalance of the regulation mechanisms and significant exhaustion of the reserve capacity of both the nervous and humoral channels of heart rate regulation.

Conclusions. 1. The absence of reliable differences between the obtained data on the work capacity girls of 17–22 years old with a low level of motor activity during the ovarian-menstrual cycle in each group of the selected types of cardiorythm regulation (vagtomy, normotonia, sympathicotonia) testifies to the insignificant degree of the influence of hormonal oscillations on the functionality of girls with low motor activity, independent of the type of vegetative regulation. The analysis of the cardiovascular system reaction to the cycle ergometric test indicates different “cost” of adaptation to muscle load in different phases of OMC for girls with different types of vegetative regulation. According to the results of cycle ergometric testing, it was found out that vagotonics are characterized by higher (130.28 ± 6.1 W), and sympathicotonics – by lower (126.1 ± 6.37 W) values of PWC_{170} as an integral indicator of the body’s functional capabilities.

2. The data of individual analysis indicate the existence of different levels of homeostasis and mechanisms of its support in patients with different types of vegetative regulation throughout the OMC. The optimal phases of physical performance for girls with vagotonic type of regulation are menstrual and premenstrual, with normotonic – menstrual and ovulatory, with sympathicotonic – postmenstrual and post-menstrual phases of OMC. Physical activities in other phases of the cycle increase the level of functional stress and can provoke pre-pathological and pathological conditions.

3. The determination of the predominant type of autonomic nervous regulation in a state of relative muscle rest allows a more objective assessment of the functional capabilities of girls aged 17–22 years with low level of motor activity and to carry out on their basis control and dosing of physical activity, which will provide optimal health effects of muscular activity without the development of tension or failure of adaptation. The obtained information can be used by specialists in the area of physical culture and sports, fitness coaches, and employees of health-improving rehabilitation centers.

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SUMMARY

DYNAMICS OF FUNCTIONAL CAPABILITIES AMONG 17-22 YEARS OLD GIRLS WITH DIFFERENT VEGETATIVE STATUS DURING THE OVARIAN-MENSTRUAL CYCLE

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The aim of the research is to study the dynamics of functional capabilities of 17–22 year old girls with different vegetative status during the ovarian-menstrual cycle. By the use of questionnaires and anthropo-physiometry, 28 girls with low motor activity and a regular (more than 3 cycles) menstrual cycle were selected, which allowed to determine the phases by calendar method. All of the surveyed people were in the main medical group of health. The modernized method of D. N. Davidenko muscular load was used. The heart rate was recorded at rest when the heart rate was reached at 150–155 beats per minute, at the end of testing and on the 5th minute of recovery, sitting on the cycle ergometer. To estimate the variability of the heart rate, a number of indicators proposed by R. M. Bayevsky were calculated to characterize the state of the vegetative nervous system. The degree of centralization of heart rate control was determined by means of a stress index, on the basis of which the group of patients was divided into subgroups depending on the type of regulation in the state of relative rest in the first (menstrual) phase of the menstrual cycle. It has been identified that vagotonics are characterized by higher, and sympathicotonics – by low values of PWC_{170} , as an integral indicator of the body's functional capabilities.

The optimal phases of physical performance for girls with vagotonic type of regulation are menstrual and premenstrual, with normotonic – menstrual and ovulatory, with sympathicotonic – postmenstrual and postovulatory phases of OMC. Physical activity in other phases of the cycle increases the level of functional stress and can provoke pre-pathological and pathological conditions.

Keywords: ovarian-menstrual cycle, variability in heart rate, hormone, cycle ergometry, functional capabilities.

РЕЗЮМЕ

ДИНАМИКА ФУНКЦИОНАЛЬНЫХ ВОЗМОЖНОСТЕЙ ДЕВУШЕК 17–22 ЛЕТ С РАЗНЫМ ВЕГЕТАТИВНЫМ СТАТУСОМ В ПЕРИОД ОВАРИАЛЬНО-МЕНСТРУАЛЬНОГО ЦИКЛА

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Исследования посвящены изучению динамики функциональных возможностей девушек 17–22 лет с разным вегетативным статусом в период овариально-менструального цикла (ОМЦ). С помощью анкетирования и антропо-физиометрии выбрано 28 девушек с низкой двигательной активностью и регулярным (более 3-х) менструальным циклом, что позволило определить фазы по календарному методу. Все обследован-

Our own experience has shown us the importance of quality of performance of every step during the investigation: from tissue sampling and processing to the immunostaining and measurement of uNK cells.

The aim of this article is to highlight the methods of uNK cell measurement in women with RPL during the last decade, to analyse our own difficulties during the endometrial tissue sampling/processing, to describe a reliable methodology of uNK cell investigation by immunohistochemical approach and summarize current trends in this direction to the establishment of the standardized protocol.

It is now well established that normal balance of immunocompetent cells in endometrium determines normal processes of implantation in the first trimester of pregnancy. 40% of total cell content in endometrium are different subpopulations of lymphocytes and the major immune cells (70-90%) among them are uNK cells [2-4]. These large, granulated lymphocytes express CD56 molecular surface marker with high density and are CD56bright and CD16⁻. The other subtype, comprising 90% of peripheral blood NK cells (bNK), express low-density CD56 and are CD56dim and CD16⁺. The latter have limited cytokine output and are primarily responsible for NK cell cytotoxicity. In contrast, uNK cells have little cytotoxic activity, but are a rich source of different cytokines and growth factors [18,19,20]. They are abundant around the spiral arteries, endometrial glands and near to extravillous trophoblast in early pregnancy. All these make uNK cells unique in terms of their tissue distribution, phenotype and function.

It has been speculated, that uNK cells are recruited from blood, however exact mechanisms for this cyclic recruitment and subsequent proliferation/ differentiation within the endometrial stroma are not well understood [3,16]. Recent data suggest that they might also be produced locally from progenitor stem cells [7]. The number of uNK cells increases dramatically in mid-luteal phase—the supposed time of implantation. It remains high during early pregnancy and comprises 70% of lymphocytes at the fetomaternal interface [9,11].

The exact function of uNK cells is unclear, although their increased number at the implantation time and their presence adjacent to the invading trophoblast suggests that uNK cells are key players in tolerogenic immune mechanisms of pregnancy [10,18]. Particularly, uNK cells regulate normal processes of trophoblast invasion through the controlled expression of receptors and secretion of chemokines [3,24]. They play also a critical role in remodelling of uterine spiral arteries and enhancing uterine vascularization by producing of angiogenic factors. Excessive angiogenesis due to high uNK cell density/activity, which also occurs during implantation, results in increased blood flow and can lead to oxidative stress and termination of pregnancy [24]. According to the recent, big multicentre study, IL-15 mediated uNK cells selectively target and clear senescent decidual cells through granule exocytosis and govern endometrial rejuvenation and remodelling [5,11]. Another group of investigators has reported that there is emerging evidence that aberrant differentiation of resident endometrial stromal cells into specialized decidual cells, due to inadequate number of uNK cells, is the hallmark of RPL [21].

Relevance of uterine NK cell research.

There is a growing interest worldwide in understanding the potential role of altered number and function of uNK cells towards the reproductive disorders, particularly RPL, recurrent implantation failure (RIF) after IVF and “Great Obstetrical Syndroms”: pre-eclampsia, pre-term delivery, fetal growth

retardation [17,18]. Intensive research is being carried out to understand better the unique immunological mechanisms between mother and fetus during successful/abnormal pregnancy and find out reliable immune markers to predict the potential risk for subsequent miscarriage. Therefore, the measurement of uNK cells acquires more significance in clinical investigational protocols of above mentioned disorders. Furthermore, there is an increasing demand from women with recurrent reproductive failure for an “uNK cell count test” and “immune treatments”, despite the fact that normal reference range and the concept of “high cell count” of uNK cells have not yet been firmly established and there is not an evidence-based immunological testing and treatments to offer them [4,18].

The literature about the link between RPL and alterations in uNK cell number is limited and controversial. Studies vary in methodology and design, their results are conflicting, investigated populations are small and heterogeneous [22,23]. Recruiting fertile controls and obtaining endometrial biopsy samples from them, due to the invasiveness and painfulness of procedure, are also problematic [2,25]. Moreover, the endometrium is a complex, dynamic tissue and its morphological structure and the number of uNK cells is highly dependent on the day of cycle. Therefore, corresponding to several researchers [10,14,18], it is important that biopsy is precisely timed and preferably taken 7 days following LH surge (LH+7). In addition, the presence of endometrial oedema, the depth from the surface epithelium and the site in uterus can all affect uNK numbers [17].

Methods of measurement of uNK cells.

There are mainly two types of assessment of NK cells: Flow cytometry and Immunohistochemistry (IHC). Flow cytometry is a traditional method of measurement of bNK cells. The advantage of this method is that it offers more detailed information about NK cell subtypes and their activation status [1,18]. It can also be used for the assessment of uNK cells, but involvement of other cell types can not be excluded, as the cell sorting is not 100% pure and mechanical and enzymatic processing of tissue can lead to loss of information of certain antigens and location of immune cells. Thus, isolation of uNK cells for this procedure is technically very difficult [1,12,18,20]. Another disadvantage of flow cytometrical assessment of uNK cells is that it requires bigger endometrial biopsy samples and immediate processing after collection. Beside this, the results may be potentially affected by several reasons, such as different protocols for preparation, labelling and gating of cell populations and transporting to the lab [20].

Immunohistochemistry evaluates uNK cells by using tissue staining technique with the monoclonal antibodies. This approach has also its limitation. First of all, taking biopsy samples is an invasive and painful procedure. Furthermore, the endometrium has complex, glandular structure and counting cells in different areas can give different results [20,25]. With IHC we can only detect the general population of CD56⁺ uNK cells and it is not possible to measure the level of their activation or detect simultaneously any subtypes of uNK cells, for example, CD56Dim, CD16⁺ cells, which are mainly involved in reproductive failure [10,20].

However, in contrast to flow Cytometry, IHC allows the visualisation of immunopositive cells within the whole endometrium and evaluation their relation to epithelial glands [18,20,22]. One of the technical advantages of IHC is that it needs smaller biopsy samples, which once fixed, can be processed later at the convenience of laboratory.

Taking into account all above mentioned reasons, assessment of uNK cells is usually done by Immunohistochemistry, however, results of many IHC studies are still controversial.

Types of variability and methods of counting of uNK cells.

High quality studies of uNK cells require reliable assessment of their density within the complex endometrial tissue. Methods of uNK cell counting vary between the mean numbers of uNK cells, the number of uNK cells as a percentage of total stromal cells [8,12,18] or as a percentage of CD45+ cells [16]. The lack of consensus in counting methods and reporting obtained results makes it difficult to compare the results of various investigations. To address this problem, one group of researchers has made an attempt to find out potential source of variability of counting uNK cells by IHC approach [15]. Precisely timed endometrial samples (LH+7-LH+9 days of cycle) were obtained from the RPL and RIF groups of women. The number of CD56+ uNK cells was expressed as a percentage of total stromal cells. Traditional cell counting (TCC) and image analysis (IA) methods were used to compare results. The authors have found relatively small intra-observer (TCC -0,94 vs. IA-0,93) and modest inter-observer (TCC-0,83 and 0,49, IA-0,43) variability. There was significant cycle-to-cycle variability in uNK cell count, but only in women with reproductive failure, which may be associated with the dysbalance of uNK cells under these specific clinical circumstances.

Concerning the minimum cell count need to be measured, authors determined that 3800 stromal cells need to be examined to achieve sufficiently reliable results, which is an equivalent to counting them in 10 high power microscope fields at x400 magnification. According to other investigators, due to the variation of uNK cell density with endometrial depth, counting CD56+ positive cells was confined to the stroma, underlying the luminal epithelium [11]. As they suggested, the characteristic feature of an abnormal decidual response is possibly excessive migration of uNK cells to subluminal parts, rather than the elevated total concentration of uNK cells.

Authors concluded, that in order to minimise the variations in counting of uNK cells between different operators/laboratories and establish their normal reference range, a single methodological IHC protocol for the measurement of uNK cells is needed.

For this purpose, one multicentre study has been carried out recently to explore why various centres, using the same IHC methodology, reported such different results and to develop a standardised method of evaluating and counting uNK cells [13].

Each of three participating centres in UK provided 5 samples of 3µm thick formalin-fixed, paraffin embedded sections of endometrial tissue. At initial analysis, the processing of tissue and immunostaining of uNK cells was carried out as per the established protocol in each centre. To determine inter-observer error, images were swapped between two centres and assessed by 3 different operators.

Variation in uNK cells number after initial evaluation.

There was considerable variation in the % CD56+/total stromal cells within the same endometrial sample reported from each centre after the initial evaluation. This was partly explained by using different counting methods-manual Vs. Image J software.

The pattern of leucocytes' distribution may also be the reason sometimes quite considerable variations-there are clustering of uNK cells, particularly around glands and blood vessels [14,17]. Researchers concluded that for a protocol to be successful it needs to be simple and easy to use and therefore, cells across the whole stroma should be counted, but not in glandular and luminal epithelium, which are easily distinguished. In addition, one of the contributing factors to this variability is a "camera

factor"-different microscope camera systems with different magnifications.

The variation in percentage of uNK cells was noticed when different staining methods with two different secondary antibody systems (in routine pathology laboratory Vs. research setting) were used. There were also differences in immunostaining patterns: samples from one centre showed clear positive staining around the cell nuclei, while others showed additional specks of immunostaining, not associated with cell nuclei. Another source of variation was due to the definition of an immunopositive cell. Therefore, it was recommended that cell nucleus must be visualized with the specific immunopositive membrane staining, to assess a cell as the positive.

Number and depth of fields for analysis

To prevent observers from the tendency ignoring the fields with less/no positive cells, researchers recommended to capture the first field near the luminal epithelial border at random and then subsequent fields-by moving one field to the left or right (skipping a field between each image), keeping the luminal edge in view, until 10 images are captured.

Not all endometrial biopsy samples contained sufficient luminal epithelium to obtain 10 adjacent x400 fields to be included, especially in RPL women (likely to be pipelle sampled). To determine the influence of depth from luminal edge on selected fields, individual up to 5x400 fields from luminal edge were chosen. The number of cells was similar when up to 4 fields away from the luminal edge were chosen. Thus, authors demonstrated that using up to 4 consecutive fields extending deeper into the stroma, will still provide valid results.

Development of an agreed protocol

After initial analysis several meetings were organized between the researchers and sources of variation were debated. It was agreed that differences in results arose from the quality of tissue fixation, processing, capture of images, selecting of areas for counting, different counting methodology and definition of immunopositive cells. As a result of intensive discussion a standardized strict protocol was adopted to minimize variations.

Several amendments were included in the protocol, among them: fixation of the tissue in a neutral, 10% buffered formalin for 24-48h in a room temperature, during sectioning ensuring that water baths are kept dust free. Images for analysis should be selected adjacent to the luminal epithelium within 5 fields, and captured digitally. Immunopositive cells should be counted using cell counter plug in Image J software, with at least 3800 total stromal cells to be counted. Endothelial cells and smooth muscle cells of blood vessels should be included in total stromal cell counting, but glandular and luminal epithelial cells -should not.

During our research work, when dealing with endometrial biopsy samples of 18 Georgian women with RPL, we have clearly seen the importance of quality of performance of above mentioned steps-from tissue sampling/processing to the capturing of images. Endometrial pipelle biopsy samples which were collected at the Centre for Reproductive Medicine "Universe", Tbilisi, Georgia, were mainly scanty and bloody, time of tissue fixation in formalin was sometimes more than 72 hours, embedding in paraffin was performed manually. Later, all of these technical errors adversely affected the further processing of endometrial tissue which was performed by one of the authors at the Placenta-Lab, University Hospital Jena, Germany. Due to the hardness of endometrial tissue during microtome sectioning, it was impossible to produce normal slides for immunostaining. Despite these difficulties, several slides were prepared, immunostained for different markers of uNK cells (CD56, CD16, CD57, CD45, CD138) and checked under the mi-

croscope. Often it was not possible to capture more than two relevant fields for counting. In many microscopic fields endometrial tissue was disrupted, presented with fragments of uneven thickness and lots of erythrocytes; luminal epithelial edge was also absent in many fields. In some areas immunopositive cells had additional positively stained spots and therefore, counting of uNK cells in many fields was not accurate and informative.

Implementation of standardized protocol

After the application of several amendments in new protocol, researchers of above mentioned study [13] have observed reduced variations in obtained counts between different centres. Additionally, images from different centre's samples were more similar to each other.

Despite the small sample size, authors achieved the main purpose of their study: to determine the reasons in variations of uNK cell counting between different researchers and identified key steps how to correct them in future investigations. As a result of this a standardisation protocol for measurement of uNK cells has been adopted, which will enable comparison of results between centres and will be very important prior its extension into clinical practice.

On the basis of the above mentioned work another multicentre retrospective study has been carried out recently in three university hospitals in UK [6]. The investigators used the recently adopted standardized protocol to determine the reference range of uNK cell percentage in precisely timed endometrial biopsy samples (LH+7) of fertile women and compared obtained results with those in women with recurrent reproductive failure.

A total of 215 women were investigated, including 97 women with unexplained RPL, 34 women with RIF and 84 fertile controls: 72 Chinese and 12 Caucasian women with proven fertility. None of the fertile controls had a history of spontaneous miscarriage. To ensure the homogeneity of specimens, they were taken only on the day 7 after LH surge in a natural cycle. Tissue processing, immunostaining and counting of CD56+ uNK cells was performed by using the standardized protocol [13].

The number of CD56+ uNK cells as % from total stromal cells for each image was calculated and the final cell count, as an average of 10 fields was reported. The new standardized methodology was verified by intra- and interobserver variability analysis, which showed acceptable levels of variation. Researchers used the 5th and 95th percentile as the lower and upper limit in fertile controls for the reference range. The nonparametric (Mann-Whitney U) test was used for comparison of CD56+ cell percentages between: 1) RPL and control groups and 2) RIF and control groups. $P < .05$ was considered significant.

uNK cell percentage in fertile women - establishment a reference range

In this study a reference range for uNK cell percentage in periimplantation endometrium was established using the 5th and 95th percentile results (to define the lower and upper limits) for a group of Chinese fertile women. This gave a reference range of uNK cell percentage as 1.2%-4.5%. The median uNK cell % from 72 Chinese controls was 2.5% (range 0.9%-5.3%). However, authors suggested that the findings may not apply to controlled ovarian stimulation cycles. The median uNK cell % from 12 Caucasian fertile women in natural cycles was 3.3% (1.1%-5.3%). There was no significant difference between Chinese and Caucasian ethnic groups.

uNK cell percentage in women with recurrent reproductive failure

In the group of women with RPL the median uNK cell percentage was 3.2% (range 0.6-8.8%), in RIF group - 3.1% (range

0.8-8.3%). In comparison with the median result of fertile controls (2.5%), there was a significantly increased % of uNK cells in both groups: in RPL - $P = .042$, in RIF - $P = .048$. Thus, this study which is consistent with many earlier studies, has shown, that uNK cell percentage was increased in women with recurrent reproductive failure, compared to fertile controls. About 22% (21/97) of women with RPL and around 29% (10/34) of women with RIF had uNK cell percentage above the upper limit, as defined by the researchers of the above mentioned study.

It is noteworthy, that all previous studies focused only on the upper limit of the reference range and limited attention had been paid to the "low uNK cell count" and its possible harmful impact to the pregnancy outcome. The working group of the above mentioned study [6], based on its proposed reference range, defined the lower limit (1.2%) and reported that significant amount of women with recurrent reproductive failure (about 38% in RPL group and 47% in RIF group) had uNK cell's level both above and below the reference range, with a major part above and a smaller proportion below this range. About 16% (16/97) of women in RPL group and 18% (6/34) of women in RIF group had uNK cell percentage below this level. However, the clinical relevance of this fact requires further investigations. Along with the use of the standardized IHC protocol and cell counting methodology for the assessment of uNK cells, this study is distinguished by several strengths as well: only precisely timed endometrial biopsy samples (LH+7) were included, significantly larger control group of 72 fertile Chinese women was organised and a reference range of uNK cell percentage was established.

Conclusion. During the last two decades heterogeneity of methods for evaluation of uNK cells, lack of reliable, standardised IHC protocol and reference range of uNK cells produced various types of investigational errors and results of many studies were incomparable. Our own experience has shown us the importance of quality of performance of every step during the investigation: from tissue sampling/processing to the immunostaining and measurement of uNK cells.

With the joint effort of different researchers in two multicentre studies standardised IHC protocol has been developed and reference range of uNK cells for fertile women has been established.

Although, not all controversial issues regarding uterine NK cells assessment and its relation to the reproductive outcome are solved and existing methods still need to be refined, the result of the above mentioned research is a very important step forward to the future investigation of uNK cells' role in unique immunological mechanisms of normal/disturbed pregnancy. Standardized methodological approach will enable researchers to minimize variability in the assessment of uNK cells, perform high quality studies with comparable results, find out meaningful immune marker for the prediction of reproductive outcome in women with a history of recurrent pregnancy loss and apply scientific findings into clinical practice. A thorough understanding of mechanisms, underlying this disorder would further advance immunological treatment innovations.

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SUMMARY

ON THE ISSUE OF STANDARDIZATION OF UTERINE NATURAL KILLER CELL MEASUREMENT IN PATIENTS WITH RECURRENT PREGNANCY LOSS

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Disturbed endometrial factor, as a result of disbalance of immune cells, is regarded as one of the most controversial immunological pathways of RPL. Uterine NK (uNK) cells are major immune cells present in endometrium and key players in tolerogenic immune mechanisms during implantation/placentation in the first trimester of pregnancy.

There is a growing interest worldwide in understanding the potential role of altered number and function of uNK cells towards the reproductive outcome. Intensive research is being carried out to find out reliable immune markers to predict the potential risk for subsequent miscarriage. Therefore, the measurement of uNK cells acquires more significance in research settings and in clinical investigational protocols as well. Furthermore, there is an increasing demand from couples with RPL for an "uNK cell count test" and "immune treatments".

Methods of measurement of uNK cells during the last decades have been challenged with many factors, that have produced various types of investigational errors and differences in results between the research centres. Researchers emphasize an urgent need for an agreed standardized protocol for the measurement of uNK cells, in order to minimize variations between the results of different centres and translate NK cell research into clinical practice.

The aim of this article is to highlight the methods of uNK cell measurement in women with RPL during the last decade, to analyse our own difficulties during the endometrial tissue sampling/processing, to describe a reliable methodology of uNK cell investigation by immunohistochemical approach and summarize current trends in this direction to the establishment of the standardized protocol.

Keywords: Natural Killer cells, endometrium, recurrent pregnancy loss, measurement method, Immunohistochemistry, standardized protocol.

РЕЗЮМЕ

К ВОПРОСУ СТАНДАРТИЗАЦИИ ОПРЕДЕЛЕНИЯ МАТОЧНЫХ НАТУРАЛЬНЫХ КИЛЛЕРОВ У ЖЕНЩИН С ПОВТОРНЫМИ ПОТЕРЯМИ БЕРЕМЕННОСТИ

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Нарушение рецептивности эндометрия (эндометриальный фактор) – самое противоречивое звено в понимании патогенеза иммунологических аспектов повторных потерь беременности (ППБ). Известно, что в толерогенных иммунных механизмах имплантации/плацентации в первом триместре беременности большая роль принадлежит маточным натуральным киллерам – uNK клеткам (uNK). Цель обзора – на основе анализа современных источников научной информации оценить потенциальную роль количества uNK клеток в репродуктивных потерях; изучить методы определения количества и функционального статуса uNK у женщин с ППБ; выделить надежные иммунные маркеры для прогнозирования риска прерывания последующей беременности. Рекомендовано uNK тестирование и иммунотерапия женщин с ППБ. Выявлена необходимость разработки стандартизированного метода оценки активности uNK клеток и их количества, а также разработка норм для здоровых фертильных женщин и женщин с патологией.

რეზიუმე

საშვილოსნოს ნატურალური კილერების განსაზღვრის სტანდარტიზაციის საკითხი ქალებში ორსულობის განმეორებითი დანაკარგებით

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ნაშრომის მიზანს წარმოადგენდა ქალებში ორსულობის განმეორებითი დანაკარგებით (ოგდ) საშვი-

ლოსნოს ნატურალური კილერების (uNK) განსაზღვრის მეთოდების მიმოხილვა, კვლევის პროცესში - ენდომეტრიუმის ბიოპტატების აღება/დამუშავებიდან მათ იმუნოჰისტოქიმიურ ანალიზამდე სირთულეების გაანალიზება, აგრეთვე იმუნოჰისტოქიმიური მეთოდით uNK უჯრედების შესწავლის თანამედროვე ტენდენციებისა და ერთიანი, შეთანხმებული სტანდარტიზირებული პროტოკოლის აღწერა.

ენდომეტრიუმის იმუნური უჯრედების დისბალანსით გამოწვეული რეცეპციულობის დარღვევა, ე.წ.ენდომეტრიალური ფაქტორი, ერთ-ერთი ყველაზე წინააღმდეგობრივი საკითხია ოგდ-ს იმუნოლოგიურ პათოგენეზში. uNK უჯრედები წარმოადგენენ ენდომეტრიუმის იმუნური უჯრედების ძირითად პოპულაციას და მთავარი მონაწილეები არიან იმპლანტაცია/პლაცენტაციის ტოლეროგენულ იმუნურ მექანიზმებში ორსულობის პირველ ტრიმესტრში. მსოფლიო მასშტაბით შეინიშნება მზარდი ინტერესი uNK უჯრედების რაოდენობისა და ფუნქციის ცვლილებების პოტენციური გავლენის შესახებ რეპროდუქციულ გამოსავალზე. ინტენსიური კვლევები ტარდება საიმედო იმუნური მარკერების აღმოსაჩენად ორსულობის მოსალოდნელი შეწყვეტის რისკის პროგნოზირებისათვის. ამიტომ, uNK უჯრედების რაოდენობის განსაზღვრა და მათი ფუნქციური სტატუსის შეფასება სულ უფრო მეტ მნიშვნელობას იძენს სამეცნიერო შრომებში კლინიკური გამოკვლევების პროტოკოლებში მათი გამოყენებისათვის. გარდა ამისა, ოგდ-ს მქონე სასოწარკვეთილი წყვილების მხრიდანაც შეინიშნება გაზრდილი მოთხოვნილება “uNK ტესტირებაზე” და იმუნურ თერაპიაზე.

ბოლო ათწლეულებში uNK უჯრედების განსაზღვრის მეთოდების არაერთგვაროვნება იწვევდა სხვადასხვა ტიპის კვლევით შეცდომებსა და მნიშვნელოვან სხვაობებს მსოფლიოს კვლევითი ცენტრების შედეგებს შორის, რაც აძნელებს მიღებული შედეგების შედარებას. დღესდღეობით, მეცნიერები მყარად თანხმდებიან იმაზე, რომ uNK უჯრედების რაოდენობის განსაზღვრისათვის აუცილებელია შეთანხმებული სტანდარტული პროტოკოლის შემუშავება კვლევის შედეგებში სხვაობების მინიმიზაციისათვის და მეცნიერული კვლევების კლინიკურ პრაქტიკაში ტრანსლაციისათვის.

ASSOCIATION OF ACE GENE POLYMORPHISM WITH THE DEVELOPMENT OF PREMENSTRUAL SYNDROME

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Premenstrual syndrome (PMS) is a complex of different symptoms (psychological, gastrointestinal manifestations, skin disorders and those connected with imbalance of water-electrolyte processes and so on) that occurs in the second phase of menstrual cycle. The variety of PMS manifestations induces the development of numerous theories of pathology [16,18]. The contribution of genetic factors in the pathogenesis of the syndrome remains to be studied.

The importance of serotonin and dopamine transporter gene polymorphisms was determined in the development of severe PMS form – premenstrual dysphoric disorder [20, 25]. The results of the scientific research indicate that such psychological symptoms as increased anxiety, depression, hopelessness, aggression and so on are associated with serotonin transporter gene polymorphism [8]. The effective therapy of PMS and premenstrual dysphoric disorder with selective serotonin reuptake inhibitors confirm the significance of serotonin transporter gene in the development of premenstrual disorders [17].

Estrogen receptor gene ESR has a certain position in the regulation behavior and mood in women. By studying of the psychological disorders that are not directly related to the changes in sex hormone levels, in particular, affective states in women with premenstrual dysphoric disorder, the importance of polymorphic variants of ESR1 gene in the control and the regulation of such behavior has been established [11,26].

Only some scientific articles are dedicated to the genetic mechanisms of PMS. In particular, it was determined that bitter taste receptor gene TAS2R38 can be associated with the presence of this syndrome [23], and CC genotype of SRD5A1 gene is the protective marker for the development of severe PMS [5]. Polymorphism of RAI1 gene can be related to the certain physical and psychological symptoms that occur during the menstrual cycle and can lead to perinatal depression [24].

Edema on extremities is very often symptom of PMS. It can be connected with the certain disorders of the regulation of renin-angiotensin-aldosterone system. That is why we can assume the value of the angiotensin-converting enzyme (ACE) gene in the development of some manifestations of PMS.

Aim of the study is to determine the frequency of polymorphism of ACE gene in patients with PMS.

Material and methods. We examined 50 women with PMS who formed basic group, 25 persons had mild PMS, 25 – severe one. 25 women without PMS were controls. Diagnosis of PMS was exhibited by the presence of cyclical manifestations of the disease in the luteal phase of the menstrual cycle on the basis of history-taking and the results of patient's self-observation diary for 2-3 menstrual cycles (R. Moos Menstrual Distress Questionnaire). The verification of severity of the diseases (mild and severe) was performed according to the Order # 676 of the Ministry of Health of Ukraine [3]. Mild PMS is the appearance of 3-4 symptoms in 2-10 days before the menstruation with significant severity 1-2 of them, severe PMS is the presence of 5-12 symptoms in 3-14 days before the menstruation, 3-4 of them are most pronounced.

Inclusion criteria: reproductive age (18-44 years), regular menstrual cycles, the presence of PMS, written consent of the patient.

Exclusion criteria: pregnancy, lactation, disorders of menstrual cycle, focal lesions of breast, abnormal uterine bleeding of unknown etiology, acute inflammatory processes in pelvic organs, tumors of uterus and ovaries of unknown etiology, endometrial hyperplasia, genital endometriosis, severe somatic pathology in the history, organic pathology of central nervous system, mental illness, hormonal tumors, diabetes mellitus, adrenal diseases, thyroid pathology, malignant tumors in the present or in anamnesis, premenstrual dysphoric disorder, women who took psychotropic medications or hormonal therapy within the last 3 months.

The clinical study was conducted on the basis of Ivano-Frankivsk City Clinical Perinatal Centre (Ivano-Frankivsk, Ukraine).

ID polymorphism of ACE gene was studied in the research laboratory at the Department of Medical Genetics in Shupyk National Medical Academy of Postgraduate Education (Kiev, Ukraine). The material for the study was peripheral blood, which was collected into tubes coated with ethylenediaminetetraacetic acid in amount 2.7 ml. Then deoxyribonucleic acid (DNA) was isolated using commercial set "DNA-sorb-B" (Institute of Epidemiology of the Ministry of Health of Russian Federation). Polymerase chain reaction was performed using the reagents of company Fermentas (Lithuania) in thermocycler «FlexCycler» (Analytik Jena, Germany). DNA amplification products were separated according to their molecular weight by electrophoresis in 2% agarose gel with the addition of ethidium bromide. Imaging was performed using a computer system Vitran.

For statistical analysis we used program Statistica 6.0 (descriptive statistics – mean, standard error; nonparametric statistics – Mann-Whitney test to compare two independent groups by a single feature; criterion χ^2 ; odds ratio (OR) and confidence interval (CI)). The difference between the values that were compared was considered significant by $p \leq 0.05$.

Results and their discussion. The average age of persons in the control group was 27.16 ± 1.09 years, the basic one – 29.42 ± 0.84 years. Also there was no difference between women of two groups in age of menarche onset (13.04 ± 0.20 and 12.94 ± 0.12 years respectively). Most of the persons had gynecological diseases in anamnesis – 21 (84.0%) controls and 46 (92.0%) patients with PMS. Special attention is ought to be paid to quantity of women in the basic group with inflammatory processes of appendages (42 persons (84.0%)), that was in 1.4 times more than among healthy individuals (15 women (60.0%); $\chi^2=4.03$, $p=0.045$; OR=3.50, 95% CI 1.16-10.52, $p=0.03$). Frequency of such diseases in patients with severe PMS (22 women (88.0%)) was in 1.47 times higher than in healthy persons ($\chi^2=3.74$, $p=0.053$; OR=4.89, 95% CI 1.15-20.79, $p=0.03$). Inflammatory diseases of low genital tract had 11 (44.0%) controls and 23 (46.0%) women in the basic group, ovarian cysts – 3 (12.0%) and 5 (10.0%) persons respectively, disorders of menstrual cycle – 7 (28.0%) and 15 (30.0%), infertility – 1 (4.0%) and 1 (2.0%), gynecological operations – 2 (8.0%) and 3 (6.0%).

Extragenital pathology was not typical for examined women. 3 (12.0%) persons of controls had chronic cholecystitis, 1 (4.0%) – pyelonephritis, 1 (4.0%) – prolapse of mitral valve.

8 (16.0%) patients with PMS suffered from pyelonephritis, 9 (18.0%) – cholecystitis and pancreatitis, 3 (6.0%) – prolapse of mitral valve.

Reproductive anamnesis in controls differs from women with PMS. Only 11 (44.0%) healthy persons were pregnant versus 35 (70.0%) patients with PMS ($\chi^2=3.72$, $p=0.053$; OR=2.97, 95% CI 1.09-8.03, $p=0.03$). 10 (40.0%) of controls had labors versus 32 (64.0%) in basic group ($\chi^2=2.98$, $p=0.08$; OR=2.67, 95% CI 0.99-7.15, $p=0.05$). There was no significant difference in above mentioned indices between women with mild and severe PMS.

The onset of the first premenstrual symptoms was at the similar age period in both PMS groups – 22.88 ± 0.99 years in patients with mild form and 23.04 ± 1.12 years – with severe one. Most of the clinical manifestations in women with PMS were connected with psychological (emotional lability, irritability, arousal) and physical disorders (peripheral edema, mastalgia, pain in bones,

muscles, lumbalgia) (Table 1). It worth to mention, that all women with PMS declared that manifestations in the second phase of menstrual cycle reduced their daily activities.

We did not find the significant difference in the distribution of ACE gene genotypes between healthy women and patients with PMS (Table 2). In both groups ID genotype predominated. Pathological DD genotype was determined more in the basic group (30.0%) than in control one (24.0%). The statistical data convincingly indicate the role of the homozygous DD genotype in the genesis of the severe PMS (52.0%). This genotype was found in 2.17 times more often in such patients compared to healthy persons ($\chi^2=3.06$, $p=0.08$; OR=3.43, 95% CI 1.02-11.47, $p=0.045$). In addition, OR value greater than one in the women with severe PMS relative to controls may indicate the association of DD genotype ACE gene with the increased risk of the development of severe form of the disease.

Table 1. Frequency of clinical manifestations in patients with premenstrual syndrome

Symptoms	Mild PMS (n=25)	Severe PMS (n=25)	Basic group, total (n=50)
Emotional lability	14 (56.0%)	20 (80.0%)	34 (68.0%)
Peripheral edema	11 (44.0%)	20 (80.0%)	31 (62.0%)
Irritability	13 (52.0%)	16 (64.0%)	29 (58.0%)
Vulgar acne, changes in skin fat	7 (28.0%)	18 (72.0%)	25 (50.0%)
Arousal	10 (40.0%)	14 (56.0%)	24 (48.0%)
Mastalgia	3 (12.0%)	14 (56.0%)	17 (34.0%)
Pain in bones, muscles, lumbalgia	4 (16.0%)	11 (44.0%)	15 (30.0%)
Fatigue, lack of energy	1 (4.0%)	13 (52.0%)	14 (28.0%)
Increase in body weight	2 (8.0%)	10 (40.0%)	12 (24.0%)
Changes in appetite and taste	4 (16.0%)	8 (32.0%)	12 (24.0%)
Sleep disturbance	8 (32.0%)	3 (12.0%)	11 (22.0%)
Drowsiness	8 (32.0%)	3 (12.0%)	11 (22.0%)
Voltage, anxiety	3 (12.0%)	6 (24.0%)	9 (18.0%)
Apathy, decline of interest	2 (8.0%)	7 (28.0%)	9 (18.0%)
Reduced muscle strength	1 (4.0%)	5 (20.0%)	6 (12.0%)
Decrease in concentration, attention	-	5 (20.0%)	5 (10.0%)
Hypersensitivity to sounds, odors	-	4 (16.0%)	4 (8.0%)
Weakness	-	3 (12.0%)	3 (6.0%)
Nausea, vomiting	-	3 (12.0%)	3 (6.0%)
Headache (migraine)	-	3 (12.0%)	3 (6.0%)
Feeling of fear, longing	-	2 (8.0%)	2 (4.0%)
Increase sweating	-	2 (8.0%)	2 (4.0%)
Dizziness	-	1 (4.0%)	1 (2.0%)

Table 2. Frequency of ID polymorphism and alleles of ACE gene in women with premenstrual syndrome and controls

Genotype/ allele	Control group (n=25)	Basic group		
		Mild PMS (n=25)	Severe PMS (n=25)	Total (n=50)
DD genotype	6 (24.0%)	2 (8.0%)	13 (52.0%)	15 (30.0%)
ID genotype	15 (60.0%)	12 (48.0%)	11 (44.0%)	23 (46.0%)
II genotype	4 (16.0%)	11 (44.0%)	1 (4.0%)	12 (24.0%)
D allele	21 (84.0%)	14 (56.0%)	24 (96.0%)	38 (76.0%)
I allele	19 (76.0%)	23 (92.0%)	12 (48.0%)	35 (70.0%)

The frequency of D allele was determined in 76.0% of persons in the basic group, which was on 8.0% less than in control one (84.0%). However, most of the carriers of D allele were found among patients with severe PMS (96.0%), which is on 12.0% higher than among healthy women. The rate of I allele was almost identical in control and basic groups – 76,0% and 70.0% respectively. However, among patients with severe PMS frequency of I allele was in 1.58 times less than in control group ($\chi^2=3.06$; $p=0.08$; $OR=0.29$, 95% CI 0.09-0.98; $p=0.045$).

Synthesis of angiotensin-converting enzyme is encoded genetically. Most of the researches demonstrate study of ID polymorphism in the 16th intron of ACE gene and its association with various diseases such as diabetic nephropathy, Alzheimer's disease, and its role in the mechanisms of the occurrence of cardiovascular phenotypes is still controversial [22].

There are some researches about ACE gene polymorphism in the implementation of gynecological pathology. It was established that A2350G polymorphism (allele G, AG genotype) of ACE gene is associated with endometriosis development but no connection with severity of the disease and treatment of infertility which is related to endometriosis [19]. According to the results of the research of G.I. Gultekin et al. ID polymorphism is not a marker of uterine leiomyoma [12]. K. Ożegowska et al. believe that persons with polycystic ovarian syndrome with DD genotype have the increased cardiovascular risk and the presence and association of metabolic disorders in women with polycystic ovarian syndrome and ACE ID polymorphism is significant [21]. While other scientists did not find the reliable link between ID polymorphism and polycystic ovarian syndrome in general population [9].

Last years no association was found between ID polymorphism of ACE gene and preeclampsia in women during pregnancy [4]. But other researches demonstrated such association [27]. Studies about the significance of ID polymorphism of ACE gene in the mechanisms of recurrent miscarriage confirmed its role in the development of this pathology [7,10]. DD and ID genotypes are usually determined in patients with idiopathic recurrent pregnancy loss [6,13,14], as well as with preterm birth [15].

There are only some scientist publications about role of ACE gene in the development of PMS. N.V. Aganezova indicates the similar distribution of genotypes of ACE gene in women with and without PMS. However, it was established that the presence of D allele in the combination with the blood renin level in the luteal phase of the menstrual cycle is most significant in the development of this pathology. In addition, the DD genotype is associated with such possible symptom of PMS as itching of the skin [1]. The reliable association of DD genotype was determined with the fact of polyuria in patients with PMS [2].

Conclusion. Our results confirm the fact of equal distribution of ACE gene genotypes between women with and without PMS with predomination of ID genotype. But women with DD genotype of ACE gene have the tendency to development of the severe form of the disease ($\chi^2=3.06$, $p=0.08$; $OR=3.43$, 95% CI 1.02-11.47, $p=0.045$).

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SUMMARY

ASSOCIATION OF ACE GENE POLYMORPHISM WITH THE DEVELOPMENT OF PREMENSTRUAL SYNDROME

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Premenstrual syndrome (PMS) is a common problem of women in reproductive age. Genetic aspects of this pathology are not completely clear. The aim of the article is devoted to the study of the frequency of ID polymorphism of angiotensin-converting enzyme gene ACE in patients with premenstrual syndrome. The object of the study were 50 women in reproductive age with the diagnosis of PMS, 25 of them had mild form of the

disease, 25 – severe one. 25 persons without PMS were controls. Polymerase chain reaction was used to study ACE gene polymorphism. We determined an equal distribution of ACE gene genotypes between women with PMS and without this pathology (DD genotype was established in 24% of controls and 30% women with PMS, ID genotype – 60% and 46% respectively, II genotype – 16% and 24%). However, DD genotype was found in 2.17 times more often in patients with severe form of the disease (52%) compared to healthy persons. Thus, women with DD genotype of ACE gene have the tendency to the development of severe PMS ($\chi^2=3.06$, $p=0.08$; OR=3.43, 95% CI 1.02-11.47, $p=0.045$).

Keywords: premenstrual syndrome, pathogenesis, ACE gene, polymorphism.

РЕЗЮМЕ

АССОЦИАЦИЯ ПОЛИМОРФИЗМА ГЕНА АСЕ С РАЗВИТИЕМ ПРЕДМЕНСТРУАЛЬНОГО СИНДРОМА

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Предменструальный синдром (ПМС) является распространенной проблемой среди женщин репродуктивного возраста. Генетические аспекты этой патологии полностью не изучены. Целью исследования явилось определение частоты ID полиморфизма гена ангиотензин-превращающего фермента (ген АСЕ) у пациентов с предменструальным синдромом. Исследованы 50 женщин репродуктивного возраста с диагнозом ПМС, 25 из них с легкой формой заболевания, 25 – с тяжелой. Контрольную группу составили 25 женщин без диагноза ПМС. Полимеразная цепная реакция использована для изучения полиморфизма гена АСЕ. Определены генотипы гена АСЕ у женщин с ПМС и без этой патологии (генотип DD встречался у 24% женщин контрольной группы и 30% женщин с ПМС, генотип ID – у 60% и 46%, соответственно, генотип II – у 16% и 24%). У пациентов с тяжелой формой заболевания генотип DD обнаружен в 2,17 раза чаще (52%), чем у здоровых женщин. Таким образом, женщины с DD генотипом гена АСЕ имеют тенденцию к развитию тяжелой формы ПМС ($\chi^2=3,06$, $p=0,08$; OR=3,43, 95% CI 1,02-11,47, $p=0,045$).

რეზიუმე

მენსტრუაციისწინა სინდრომის განვითარებასთან დააკავშირებული ACE გენის პოლიმორფიზმი

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სუსდ „ივანო-ფრანკოესკის ეროვნული სამედიცინო უნივერსიტეტი“, უკრაინა

მენსტრუაციისწინა სინდრომი (მწს) წარმოადგენს გავრცელებულ დაავადებას რეპროდუქციული ასაკის ქალებში. ამ პათოლოგიის გენეტიკური საფუძველი არ არის ბოლომდე შესწავლილი. კვლევის მიზანს წარმოადგენდა ანგიოტენზინ-გარდაამქმნელი ფერმენტების გენის (ACE გენი) ID პო-

ლიმორფიზმის სისხირის შესწავლა პაციენტებში მენსტრუაციისწინა სინდრომით. გამოკვლეულია რეპროდუქციული ასაკის 50 ქალი დიაგნოზით მწს, რომელთაგან 25 აღმოაჩნდა დაავადების მსუბუქი ფორმა, 25 – მძიმე ფორმა. საკონტროლო ჯგუფი შეადგინა 25 ქალმა მწს დიაგნოზის გარეშე. ACE გენის პოლიმორფიზმის შესწავლის მიზნით გამოყენებული იყო პოლიმერაზული ჯაჭვური რეაქცია. დადგენილია ACE გენების გენოტიპების თანაბარი განაწილება ქალებში მწს-ით და ამ პათოლოგიის გარეშე (გენოტი-

პი DD გამოვლინდა 24%-ში საკონტროლო ჯგუფში და 30% ქალებში დიაგნოზით მწს, გენოტიპი ID – 60% და 46%, შესაბამისად, გენოტიპი II – 16% და 24%). მწს-ის მძიმე ფორმით დაავადებულ პაციენტებში გენოტიპი DD გამოვლენილია 2,17-ჯერ უფრო ხშირად (52%), ვიდრე ჯანმრთელ ქალებში. მიღებული მანკენებლებიდან გამომდინარე, ACE გენის გენოტიპის მქონე ქალებში არსებობს მწს მძიმე ფორმის განვითარების ტენდენცია ($\chi^2=3,06$, $p=0,08$; $OR=3,43$, 95% CI 1,02-11,47, $p=0,045$).

NEW GENETIC MARKERS ASSOCIATED WITH SUSCEPTIBILITY TO EXFOLIATION SYNDROME AMONG GEORGIAN POPULATION

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Exfoliation syndrome (XFS) is an age-related systemic disorder of the extracellular matrix characterized by excessive production and progressive accumulation of extracellular fibrillar material (XFM) in the anterior chamber of the eye on the lens, iris, ciliary body, as well as other intraocular structures. XFS is the most frequent reason of intra- and postoperative complications in cataract surgery as a result of tissue laxity from XFM deposition. It is also the most common cause of secondary exfoliative glaucoma (XFG) which results from accumulation of XFM in the trabecular meshwork, leading to elevated intraocular pressure which eventually damages the optic nerve.

Sixty to 70 million people are affected by XFS worldwide [18]. Patients with XFS have twofold increased risk of converting ocular hypertension to XFG [11] which in contrast to primary open angle glaucoma is characterized by much more severe clinical course, more fluctuation of IOP, less response to conservative treatment, faster progression of optic nerve atrophy and visual field deterioration, more frequent need of surgical treatment.

XFS is a heritable condition. This fact is long known from familial aggregation studies. Genetic markers of XFS are also actively investigated. XFS and XFG patients are found to have three high-risk SNPs of LOXL1 gene on chromosome 15: rs2165341, rs1048661 and rs3825942 [1,4-10,18-20]. LOXL1 codes for lysyl oxidase which is a member of copper-dependent enzymes, which plays role in genesis, stabilization and remodeling of elastic fibers [12]. It catalyses deamination of lysyl remnants of tropoelastin, which is the most important reaction in elastogenesis [18,20]. It is synthesized as a precursor which undergoes glycosylation in Golgi complex and is secreted to extracellular space, where it is proteolytically degraded to become an active enzyme [21]. In XFS patients hyperproduction, cross-linking and aggregation of elastic fibers occurs where lysyl oxidase plays a major role [18].

Recently, genome-wide association studies showed strong association between 6 other genetic loci (CACNA1A, POMP, TMEM136, AGPAT1, RBMS3, and SEMA6A) and increased risk of XFS [2]. Interestingly, all these genes encode proteins which could point to different mechanisms of disease develop-

ment. These are calcium channel subunit, proteasome maturation protein, semaphorin, involved in neuronal development, to name a few. As XFS is very common in Georgia, studying its genetic markers in our population could give some insight in the disease pathogenesis.

Material and methods. Self-reported Georgian subjects were recruited between 2015 and 2017 at an ophthalmology tertiary care center. Patients underwent detailed ophthalmic examination, including Humphrey 24-2 perimetry and optic nerve head (ONH) evaluation to exclude glaucoma. Subjects with XFS exhibited deposits of exfoliation material on pupillary margin or lens capsule upon slit lamp examination. Subjects with glaucoma had characteristic changes of ONH, including increased vertical cup-to-disc ratio, retinal nerve fiber layer thinning, neuroretinal rim notching or hemorrhages. Patients over age 50 with evident XFS and XFG were included into the study. The control group comprised patients over age 60 with no evidence of exfoliations and glaucoma upon clinical examination. Patients having uveitis and neovascular glaucoma were excluded from the study.

The ethical approval of this study was obtained from David Tvildiani Medical University Ethics Committee. All study procedures were adherent to principles stated in Declaration of Helsinki on Biomedical Research Involving Human Subjects. After signing informed consent, subjects underwent blood sampling. We collected 5 ml of peripheral blood and refrigerated in EDTA-coated tubes before use. DNA extraction was performed using a DNA extraction kit according to manufacturer's protocol. We performed principal-component analysis to assess the degree of genetic stratification and population substructure for all samples. Genetic outliers were excluded from the study. Genome-Wide Association Study (GWAS) was performed using Illumina OmniExpress Microarray. More than 680 000 SNPs were analyzed for association to XFS.

Statistical analysis of our case-control association study was performed using a χ^2 test (Pearson correction). Relative risk association was estimated by calculating odds ratios (OR) along with 95% confidence intervals (CIs). $p < 0.05$ was considered statistically significant.

Results and their discussion. One hundred and thirty-two patients with XFS were included in our study (Table 1). Seventy-three (56%) patients were female and 59 (44%) patients were male. Mean age was 73.7 (± 6.4) years. One hundred and fourteen (86%) patients were diagnosed with XFS only, 18 (14%) had XFG. The control group comprised 199 patients without any clinical evidence of XFS or XFG. One hundred and thirty control patients (65%) were female and 69 (35%) patients were male. Mean age was 70.8 (± 7.3) years.

We identified six genes previously reported in association to XFS: CACNA1A rs4926244, POMP rs7329408, TMEM136 rs11827818, AGPAT1 rs3130283, RBMS3 rs12490863 and SEMA6A rs10072088. The results are shown in Table 2.

Among six genes studied, SEMA6A, POMP and CACNA1A were found to be associated with XFS in our population. Allele frequencies of SEMA6A were significantly different when comparing cases and controls and associated with XFS ($p=0.001$). High-risk allele A did increase disease susceptibility by 80%. It was present in 81% of affected individuals, 65% of them were homozygous. Heterozygotes had almost 2-fold increased risk ($p=0.001$; OR=1.8; 95% CI: 1.2676 to 2.6973), whereas, homozygotes had the risk of disease development up to 4 times higher ($p=0.001$; OR= 4.0; 95% CI: 1.1531 to 13.9903) than normal

individuals. Interestingly, allele A was found in up to 70% of normal individuals and half of them were homozygous.

High-risk allele A of POMP was found only in 16% of XFS cases. The likelihood of disease development rose up to 60% in affected individuals ($p=0.005$; OR= 1.6; 95% CI: 0.9931 to 2.5634). Heterozygotes did not show any increased risk of disease development ($p=0.7$; OR=1.5; 95% CI: 0.0937 to 24.3786) and homozygotes had up to 70% ($p=0.06$; OR= 1.7; 95% CI: 1.0217 to 2.8713) higher risk when compared to individuals who were not carrying a high-risk allele. Normal individuals carried allele A in 10% of cases and only 0.5% of them were homozygous.

We identified allele G of CACNA1A as high-risk. It was present in about 20% of both affected and normal individuals and only the homozygotes carried an increased risk of disease development up to 3 times ($p=0.05$; OR= 3.15; 95% CI: 0.9275 to 10.6658).

For the other three genes tested (TMEM136 rs11827818, AGPAT1 rs3130283, RBMS3 rs12490863) we did not observe any statistically significant differences in allele frequencies. Previously reported high-risk alleles were present both in controls and affected individuals in 10-15% of cases respectively and they did not confer any risk of disease development.

Table 1. Demographic characteristics of XFS patients and controls in the Georgian population

	Total	Male	Female
XFS	132	59 (44%)	73 (56%)
XFS only	114 (86%)	49 (43%)	65 (57%)
XFG	18 (14%)	10 (55%)	8 (45%)
Controls	199	69 (35%)	130 (65%)

Table 2. Allele and Genotype Frequencies of SEMA6A, CACNA1A and POMP genes in XFS and healthy subjects

SNP		Controls % (n=199)	XFS % (n=132)	P value	OR (95% CI)
SEMA6A-rs10072088					
Allele	A	70.4	81.4	0.001	1.8 (1.2676 to 2.6973)
	G	29.7	18.6	0.001	0.5 (0.3299 to 0.8166)
Genotype	GA	42.3	32.5	0.002	1.9 (1.2246 to 3.0316)
	AA	49.2	65.2	0.004	4.0 (1.1531 to 13.9903)
	GG	8.5	2.3	0.004	0.2 (0.0715 to 0.8672)
	Total	98/84/17 (AA/GA/GG)	86/3/43 (AA/GA/GG)		
POMP-rs7329408					
Allele	A	9.8	14.8	0.04	1.6 (0.9931 to 2.5634)
	G	90.2	85.2	0.05	0.6 (0.3901 to 1.0069)
Genotype	AG	18.6	28	0.7	1.5 (0.0937 to 24.3786)
	AA	0.5	0.8	0.05	1.7 (1.0217 to 2.8713)
	GG	80.9	71.2	0.05	0.6 (0.3483 to 0.9788)
	Total	161/37/1 (GG/AG/AA)	94/37/1 (GG/AG/AA)		
CACNA1A-rs4926244					
Allele	G	21.21	18.34	0.4	1.2 (0.8122 to 1.7690)
	A	78.8	81.7	0.4	0.8 (0.5653 to 1.2313)
Genotype	AG	42.3	32.5	0.7	1.08 (0.6801 to 1.7043)
	GG	8.5	2.3	0.05	3.15 (0.9275 to 10.6658)
	AA	49.2	65.2	0.05	0.3 (0.0938 to 1.0782)
	Total	40/84/8 (AG/AA/GG)	65/130/4 (AG/AA/GG)		

Table 3. Allele and Genotype Frequencies of SEMA6A and POMP genes in XFG and healthy subjects

SNP		Controls % (n=199)	XFS % (n=18)	P value	OR (95% CI)
SEMA6A-rs10072088					
Allele	A	70.4	86	0.04	3.4 (1.1663 to 9.7455)
	G	29.7	14	0.04	0.2(0.1026 to 0.8574)
Genotype	AA	49.2	65.2	0.002	3.6 (1.1474 to 11.3402)
	GA	42.3	32.5	0.002	1.9 (1.2246 to 3.0316)
	GG	8.5	2.3	0.004	0.2 (0.0715 to 0.8672)
	Total	98/84/17 (AA/GA/GG)	14/3/1 (AA/GA/GG)		
POMP-rs7329408					
Allele	A	9.8	22	0.02	2.7 (0.9931 to 2.5634)
	G	90.2	78	0.02	0.4 (0.3901 to 1.0069)
Genotype	AA	0.5	5.6	0.03	11.6(0.6972 to 194.5768)
	GA	18.6	33.4	0.05	2.7 (0.0937 to 24.3786)
	GG	80.9	61	0.05	0.4 (0.3483 to 0.9788)
	Total	161/37/1 (GG/GA/AA)	11/6/1 (GG/GA/AA)		

Among the six genes studied, only SEMA6A and POMP were associated with XFG in our population. Allele frequencies of SEMA6A were significantly different when comparing cases and controls and associated with XFG ($p=0.04$). High-risk allele A increased disease susceptibility more than 3 times ($p=0.04$; OR= 3.4; 95% CI: 1.2676 to 2.6973). It was present in 86% of our patients, 65% of them were homozygous. Heterozygotes had twice increased risk ($p=0.002$; OR= 1.9; 95% CI: 1.2246 to 3.0316), whereas, homozygotes had the risk of disease development up to 4 times higher ($p=0.002$; OR= 3.6; 95% CI: 1.1474 to 11.3402) compared to controls.

High-risk allele A of POMP was found in 22% of XFG cases. The likelihood of disease development rose up to 3 times in affected individuals ($p=0.02$; OR= 2.7; 95% CI: 0.9931 to 2.5634). Heterozygotes showed 3-fold increased XFG risk ($p=0.05$; OR= 2.7; 95% CI: 0.0937 to 24.3786) and homozygotes had up to 11 times ($p=0.05$; OR= 11.6; 95% CI: 0.6972 to 194.5768) higher risk when compared to individuals who were not carrying a high-risk allele.

The LOXL1 gene is so far the best characterized gene predisposing individuals to XFS development. Recently, a huge Genome-wide Association study found several different loci in association with the disease. We tested these genes in our population and determined the association of the three of them with XFS. These are SEMA6A, POMP and CACNA1A.

CACNA1A encodes for the alpha 1A subunit of the type P/Q voltage-dependent calcium channel. Calcium channels play a key role in a cell's ability to generate and transmit electrical signals. Previous electron microscopy studies on human XFS eyes showed the presence of high calcium concentration in XFS fibrils [3]. On the other hand, it is well known that fibrillin utilizes calcium to form stable aggregates [17]. Thus, it can be hypothesized that altered function of a calcium channel could lead to alterations of calcium concentrations that may facilitate the formation of XFS aggregates.

POMP encodes proteasome maturation protein. It functions as a proteolytic enzyme for newly assembled dysfunctional proteins. Its expression is downregulated in XFS [2], as shown in recent studies. Therefore, it can be one of the factors causing pathologic aggregation of XFM. A single nucleotide deletion causes keratinizing skin disorders and ichthyosis.

Semaphorin, encoded by SEMA6A is found in developing neural cells and is involved in axonal guidance. SEMA6A mutations are observed in several neurological diseases. It is interesting to see, that XFG risk is increased 3.6-fold in homozygous individuals.

Our data suggest several new mechanisms of XFS development. Further studies are necessary to define exact mechanisms leading to XFS and especially XFG. The limitation of our study was a very small subgroup of XFG patients, which therefore, could not reflect the whole picture.

Conclusions. We studied six proposed genetic markers of XFS in Georgians, where the disease is extremely prevalent in general population. We identified high-risk alleles of SEMA6A, POMP and CACNA1A which theoretically can contribute to disease development in Georgians. The other three genes possibly play no role in XFS pathogenesis. Only SEMA6A and POMP are associated with XFG in our population.

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SUMMARY

NEW GENETIC MARKERS ASSOCIATED WITH SUSCEPTIBILITY TO EXFOLIATION SYNDROME AMONG GEORGIAN POPULATION

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The aim of this study was to identify susceptibility variants of CACNA1A, POMP, TMEM136, AGPAT1, RBMS3, and SEMA6A genes for Exfoliation Syndrome (XFS) and Exfoliation Glaucoma (XFG) by a case-control association study approach among Georgian population.

Self-reported Georgian subjects were recruited between 2015 and 2017 at a specialized ophthalmic center. Patients underwent detailed ophthalmic examination to diagnose or exclude Exfoliation Syndrome and Exfoliation Glaucoma. Patients underwent peripheral blood sampling. Genome-Wide Association Study (GWAS) was performed using Illumina OmniExpress Microarray (USA).

One hundred and thirty-two XFS patients (including XFG-affected individuals) and 199 healthy subjects were included into the study. Six genes CACNA1A rs4926244, POMP rs7329408, TMEM136 rs11827818, AGPAT1 rs3130283, RBMS3 rs12490863 and SEMA6A rs10072088 variants were identified. The A alleles of SEMA6A and POMP genes are likely the risk factors of disease development in Georgians with $p=0.001$; OR= 1.8, 95% CI 1.2676 to 2.6973 and $p=0.001$; OR=1.6, 95% CI 0.9931 to 2.5634, respectively. SEMA6A homozygotes have 4 times greater risk compared to normal individuals, with $p<0.004$; OR=4.0, 95% CI 1.1531 to 13.9903. The G allele of CACNA1A in homozygous state increases the risk up to 3-fold with $p<0.05$, OR=3.15, 95% CI 0.9275 to 10.6658. The A alleles of SEMA6A and POMP increased XFG susceptibility more than 3 times ($p=0.04$; OR= 3.4; 95% CI: 1.2676 to 2.6973 and $p=0.02$; OR= 2.7; 95% CI: 0.9931 to 2.5634, respectively).

Three high-risk genes have been identified in connection to XFS in Georgian population. Two genes are relevant to XFG. Three other previously described genes are not associated with the disease development.

Keywords: exfoliation syndrome, exfoliation glaucoma, GWAS.

РЕЗЮМЕ

НОВЫЕ ГЕНЕТИЧЕСКИЕ МАРКЕРЫ, АССОЦИИРОВАННЫЕ С ЭКСФОЛИАТИВНЫМ СИНДРОМОМ СРЕДИ ГРУЗИНСКОЙ ПОПУЛЯЦИИ

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Целью исследования явилось определение связи генов CACNA1A, POMP, TMEM136, AGPAT1, RBMS3 и SEMA6A с эксфолиативным синдромом.

В 2015-2017 гг. исследованы этнические грузины - 132 пациента с эксфолиативным синдромом (основная группа, включающая 18 больных эксфолиативной глаукомой) и 199 здоровых лиц (контрольная группа). После информированного согласия, у пациентов забиралась периферическая кровь. Исследование генетического материала производилось с помощью чипов Illumina OmniExpress (США).

Исследованы 6 генов CACNA1A rs4926244, POMP rs7329408, TMEM136 rs11827818, AGPAT1 rs3130283, RBMS3 rs12490863 и SEMA6A rs10072088, 3 из них оказались ассоциированными с синдромом: аллели А генов SEMA6A и POMP оказались носителями высокого риска развития эксфолиативного синдрома ($p=0.001$; $OR=1.8$, 95% CI 1.2676 to 2.6973 и $p=0.001$; $OR=1.6$, 95% CI 0.9931

to 2.5634, соответственно). У гомозиготных носителей аллелей высокого риска генов SEMA6A и CACNA1A риск развития заболевания был в 3-4 раза больше ($p<0.004$; $OR=4.0$, 95% CI 1.1531 to 13.9903 и $p<0.05$, $OR=3.15$, 95% CI 0.9275 to 10.6658, соответственно). Риск развития эксфолиативной глаукомы повышался в 3 раза у носителей аллелей А генов SEMA6A и POMP ($p=0.04$; $OR=3.4$; 95% CI: 1.2676 to 2.6973 и $p=0.02$; $OR=2.7$; 95% CI: 0.9931 to 2.5634, соответственно)

У исследованных пациентов обнаружено три полиморфизма, ассоциированных с эксфолиативным синдромом и два полиморфизма, ассоциированных с эксфолиативной глаукомой. Три других, предполагаемых гена не оказались связанными с данным состоянием.

რეზიუმე

ექსფოლიაციურ სინდრომთან ასოცირებული ახალი გენეტიკური მარკერები ქართულ პოპულაციაში

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კვლევის მიზანს წარმოადგენდა CACNA1A, POMP, TMEM136, AGPAT1, RBMS3, და SEMA6A გენების მაღალი რისკის პოლიმორფიზმების კავშირის განსაზღვრა ექსფოლიაციური სინდრომის განვითარებასთან პაციენტებში. კვლევაში ჩართული იყო 132 პაციენტი და 199 ჯანმრთელი ეთნიკურად ქართველი ინდივიდი, რომლებიც დეტალური ოფთალმოლოგიური კვლევის შედეგად განაწილდნენ ექსფოლიაციური სინდრომის (მათ შორის ექსფოლიაციური გლაუკომის) მქონე პაციენტებისა და საკონტროლო ჯგუფებში. ინფორმირებული თანხმობის ხელმოწერის შემდეგ ხდებოდა პერიფერიული სისხლის აღება და გენეტიკური მასალის გამოყოფა. სრული გენომის შესწავლა განხორციელდა Illumina OmniExpress (აშშ) მიკროჩიპების საშუალებით.

შესწავლილია CACNA1A rs4926244, POMP rs7329408, TMEM136 rs11827818, AGPAT1 rs3130283, RBMS3 rs12490863 და SEMA6A rs10072088 გენების კავშირი ექსფოლიაციურ სინდრომსა და გლაუკომასთან. SEMA6A და POMP გენების A ალელები აღმოჩნდნენ

მაღალი რისკის მატარებელნი ქართულ პოპულაციაში ($p=0.001$; $R=1.8$, 95% CI 1.2676 to 2.6973 და $p=0.001$; $OR=1.6$, 95% CI 0.9931 to 2.5634, შესაბამისად). SEMA6A პომოზიგოტებს აღმოაჩნდათ ყველაზე მაღალი რისკი, ჯანმრთელ ინდივიდებთან შედარებით ($p<0.004$; $OR=4.0$, 95% CI 1.1531 to 13.9903). ასევე, CACNA1A გენის G ალელის პომოზიგოტ მატარებლებს დაავადების განვითარების რისკი ეზრდებოდათ 3-ჯერ ($p<0.05$, $OR=3.15$, 95% CI 0.9275 to 10.6658). TMEM136, AGPAT1 და RBMS3 გენებთან კავშირი არ აღმოჩნდა. SEMA6A-სა და POMP-ის A ალელები ექსფოლიაციური გლაუკომის რისკს ზრდიდნენ 3-ჯერ ($p=0.04$; $OR=3.4$; 95% CI: 1.2676 to 2.6973 და $p=0.02$; $OR=2.7$; 95% CI: 0.9931 to 2.5634, შესაბამისად).

ეთნიკურად ქართველ ინდივიდებში გამოვლინდა ექსფოლიაციურ სინდრომთან ასოცირებული სამი და ექსფოლიაციურ გლაუკომასთან ასოცირებული მაღალი რისკის მქონე გენი. სამი ადრე აღწერილი გენი არ აღმოჩნდა დაავადებასთან კავშირში.

СТРЕСС КАК ФАКТОР-ПРЕДИКТОР РАЗВИТИЯ ПЕРИИМПЛАНТИТА (ОБЗОР)

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В настоящее время все больше и больше людей желают иметь красивую здоровую улыбку. В век современных технологий, применяемых на стоматологическом приеме, добиться этого результата задача несложная. При частичном, и даже полном отсутствии зубов у пациентов и врачей-стоматологов все большую популярность приобретает имплантация. Потерянный зуб, может не только испортить улыбку, но и доставляет множество неудобств и проблем. Особенно это касается жевательных коронок, отсутствие которых усиливает нагрузку на противоположные и соседние зубы. В результате они расшатываются, изменяется прикус, быстрее развивается кариес. Решить эту проблему можно с помощью имплантации, которая на сегодняшний день является новейшей прогрессивной методикой по восстановлению утраченных зубов [5].

Имплантация – это операция по установке в челюстную ткань специального стоматологического штифта, который заменяет корень, затем на штифт закрепляется коронка, и полученная прочная конструкция полностью заменяет потерянный зуб. Имплантат в виде корня зуба изготавливается из высококачественного титана. Используемый материал является гипоаллергенным и не вызывает у пациентов никаких осложнений. После приживления искусственного корня на него устанавливается абатмент – промежуточная часть между имплантатом и коронкой. Примерно 99% имплантатов успешно приживаются, не вызывая каких-либо осложнений. Имплантаты применяются не только для эстетических целей, но и для восстановления жевательной функции зубов, распределения равномерной нагрузки на весь зубной ряд, предупреждения развития воспалительных и дистрофических заболеваний пародонта и височно-нижнечелюстного сустава. Несмотря на то, что установка имплантата в настоящее время не связана с трудностями, очень часто после дентальной имплантации развиваются осложнения. Одним из распространенных осложнений является периимплантит [2].

Периимплантит – воспаление тканей вокруг остеоинтегрированного имплантата, приводящее к прогрессирующей убыли опорной кости. Согласно данным клинических исследований, при использовании различных современных имплантационных систем периимплантит развивается в 12-43% случаев [12] и является одной из частых причин отторжения дентального имплантата. В отличие от периимплантита мукозит является воспалением прилегающего к имплантату участка слизистой без признаков убыли костной ткани. Дентальная имплантология активно развивающееся направление современной стоматологии. Количество пациентов, имеющих как съемные, так и несъемные протезы с опорой на имплантаты, неуклонно растет. В связи с этим вопросы повышения успешности дентальной имплантации и профилактики осложнений приобретают особую значимость [22].

Многочисленные общие и местные факторы-предикторы развития периимплантитов и мукозитов можно разделить на два типа [6]– общие и местные. К общим факторам относятся: 1. Токсический и аллергический фактор – аллергическая реакция на медикаментозные и химические раздражители.

2. Нейрогенный фактор – за счет симпатго-адреналовой одномоментной реакции: стресс, беспокойность во время сна, нервно-психические потрясения, негативные эмоции.

3. Иммунный фактор – по типу поздней иммунологической реакции с цитотоксическим эффектом.

4. Микроциркуляторные нарушения – на фоне снижения скорости базального кровотока и вазоконстрикции сосудов.

Местные факторы – это неудовлетворительная гигиена полости рта.

II тип факторов является провоцирующим, к ним относятся факторы, зависящие от действий врача и от действий пациента. При этом в ближайшем послеоперационном периоде осложнения, как правило, обусловлены техническими погрешностями при проведении хирургического этапа имплантации и протезирования, а в отдаленном периоде – несоблюдением пациентом гигиенических норм. Среди врачебных ошибок, являющихся причиной развития периимплантита следует отметить нарушение правил асептики и антисептики; неправильная оценка факторов риска и подбора и установки (позиционирование) внутрикостной части имплантата, формирователя десны, абатмента; неправильно изготовленные ортопедические конструкции (коронки, протезы), приводящие к перегрузке и хронической травме пародонта. Наиболее типичными факторами, вызывающими периимплантит, являются формирование поддесневой гематомы с ее последующим нагноением; несоответствие костного ложа размерам имплантата, вследствие чего развивается подвижность конструкции; разрушение костной ткани, вызванное чрезмерным усилием вкручивания имплантата (более 45 Н/м); неадекватное ушивание операционной раны, наличие микрозачерков между имплантатом и абатментом.

Что касается использования для протезирования имплантационных систем сомнительного качества, эта причина периимплантита встречается очень редко и может быть отнесена к сфере медицинской ответственности. В этом случае осложнение может быть связано с низким качеством титанового сплава, недоработанным дизайном имплантата, применением имплантационных систем-подделок [2].

Наиболее частой причиной периимплантита является неадекватный уход пациента за естественными зубами, имплантатом и фиксированным на нем протезом, игнорирование профилактических осмотров и профессиональной гигиены полости рта. Конструктивные особенности имплантатов предрасполагают к образованию зубного налета и зубного камня, что, в свою очередь, вызывает воспаление окружающих тканей и периимплантит.

В группе риска по развитию периимплантита находятся курильщики, пациенты с заболеваниями пародонта (гингивит, пародонтит, пародонтоз), бруксизмом, иммунными нарушениями, сахарным диабетом. На успешность интеграции имплантата влияет выбор тактики имплантации – одноэтапной или классической, показания и противопоказания к которой должны быть учтены при планировании лечения [18].

Вышеперечисленные местные факторы – предикторы развития мукозитов, являются легко контролируемыми у

компетентных пациентов. Что касается общих факторов, то аллергический компонент выявить не представляет особой сложности с помощью проведения аллергологических проб. Стрессовое состояние является причиной многих заболеваний, в том числе и стоматологических. Согласно данным Всемирной организации здравоохранения (ВОЗ), пародонтозом болеют более 90% взрослого населения планеты. По данным учёных швейцарского университета в Цюрихе, такие заболевания прямо связаны со стрессом [1,7,11].

Стресс является неспецифическим ответом организма на любое предъявленное к нему требование. Известно, что человек испытывает стресс не только когда злится, переживает или раздражается, но и при положительных эмоциях. Такой вид стресса считается эустрессом, т.е. хорошим стрессом, который исходит от позитивных событий в жизни – радость при встрече вызывает такой же стресс как и раздражение. Независимо от того, чем вызвана стрессовая ситуация, человек испытывает сначала тревогу (это может быть ещё и паника или же приятное волнение), а после этого уже происходит целый ряд различных приспособительных реакций организма. Малые стрессовые реакции отлично тренируют организм, однако постоянные являются причиной заболеваний, так как приспособляющийся механизм быстро истощается. Современное общество, к сожалению, живет в постоянном стрессе. Следует отметить, что у большинства (90%) визит к стоматологу также вызывает стрессорное состояние [14,16]. Во время стрессовых ситуаций человеческий организм вырабатывает кортизол – гормон стресса, высокий уровень которого часто приводит к воспалительным процессам тканей пародонта — комплекса тканей, окружающих зуб и предоставляющих надёжную фиксацию в челюсти.

Стресс влияет на соблюдение правильной гигиены полости рта. Данные исследования *Neuroendocrinology Letters* [13] сообщают о том, что именно стресс влияет на гигиенические ежедневные привычки 565 людей. Иначе говоря, человек начинает меньше внимания отдавать чистке зубов, перестаёт пользоваться ополаскивателями для полости рта, зубные нити в тот момент, когда подвержен стрессу. Несомненно, внимательное отношение к своему психическому эмоциональному состоянию очень важно, как и грамотное поведение во время стрессовых ситуаций, так как полное их избежание невозможно.

Одним из факторов, способствующих росту неинфекционных заболеваний, в том числе и стоматологических, является высокий уровень психоэмоциональной напряженности в жизни современного человека. Несмотря на огромный поток информации, посвященный проблемам стресса, его роль в генезе стоматологических заболеваний, а в частности периимплантитов изучена недостаточно. Снижение местных и общих механизмов защиты от повреждающих факторов, нарушение метаболических процессов в пародонтальном комплексе, как правило, происходит в случаях нервно-соматических заболеваний, нарушениях обмена веществ, частых и длительных стрессах [2].

Влияние стресса на развитие стоматологических заболеваний и их осложнений не вызывает сомнений, однако, раннее выявление данного состояния и коррекция существующей схемы патогенетической и симптоматической терапии стоматологического больного позволяет предотвратить развитие периимплантитов и мукозитов.

Одним из объективных достоверных методов диагностики психологического стресса является лазерная доплеровская флоуметрия (ЛДФ). Данный метод является доступ-

ным, неинвазивным, способным наблюдать за состоянием организма *in vivo* в динамике лечения [3,9].

ЛДФ проводится с помощью приборов, предназначенных для этих целей. В России наиболее часто используются разные модификации прибора «ЛАКК» – анализатора лазерного для оценки микроциркуляции крови. В качестве датчика в приборах ЛАКК применяется световой зонд, выполненный из трёх моноволокон. Одно волокно используется для доставки лазерного излучения от прибора к исследуемому объекту, два других волокна являются приёмными для рассеянного в ткани лазерного излучения [10]. Доставленное по волокнам рассеянное излучение детектируется двухканальным фотоприёмным устройством. Электрические сигналы с фотоприёмного устройства поступают в блок обработки анализатора, где происходит выделение в зарегистрированном сигнале доплеровского сдвига частоты. После аналоговой обработки формируется выходной сигнал, пропорциональный произведению множителей: средней скорости движения эритроцитов и их концентрации в зондируемом объёме ткани [4].

После регистрации ЛДФ-грамм на монитор выводились показатели амплитуд сигнала на выходе прибора: величина среднего потока перфузии крови – M в интервале времени регистрации, измеряется в перфузионных единицах (пф.ед), параметр σ («флак») – среднее колебание перфузии относительно среднего значения потока крови M , измеряется в перфузионных единицах и интегральный показатель вариаций – K_v данного процесса, расчетные параметры, которые позволяют проводить общую оценку состояния гемомикроциркуляции (в %) [8].

Более детальный анализ функционирования микроциркуляторного русла проводится на втором этапе обработки ЛДФ-грамм базального кровотока при исследовании структуры ритмов колебаний перфузии крови – амплитудно-частотный анализ (АЧС) колебаний перфузии. По величинам амплитуд колебаний микрокровотока в конкретных частотных диапазонах возможно оценивать состояние функционирования определенных механизмов контроля перфузии [20].

Микроциркуляторное русло находится под многоуровневым контролем, который организован через систему обратной связи. С помощью ЛДФ-метрии можно оценить влияние активных (эндотелиальный, нейрогенный и миогенный механизмы) и пассивных (пульсовая и дыхательная волна) факторов. Изменения вышеперечисленных факторов вызывают модуляцию перфузии. Активные факторы напрямую оказывают влияние на микроциркуляторную систему. К ним относятся эндотелиальные – VLF – колебания, миогенные – LFM и нейрогенные LFN – колебания.

Факторы, вызывающие колебания капиллярного кровотока вне микроциркуляторной системы, относятся к пассивным (амплитуды дыхательной и пульсовой волны).

Амплитуда пульсовой волны (CF), попадает в микроциркуляторное русло со стороны артерий, является параметром, который изменяется в зависимости от состояния тонуса резистивных сосудов. Очевидно, что при снижении указанного сосудистого тонуса, увеличивается объем притока артериальной крови в микроциркуляторное русло, модулированной пульсовой волной [21].

Дыхательная волна (HF) обусловлена динамикой венозного давления при легочной механической активности. Увеличение амплитуды дыхательной волны указывает на снижение микроциркуляторного давления. Ухудшение оттока крови из микроциркуляторного русла может сопрово-

ждаться увеличением объема крови в веноулярном звене, что приводит к росту амплитуды дыхательной волны в ЛДФ – грамме.

Пассивные факторы вызывают продольные колебания кровотока, отражающие периодические изменения объема крови. Поперечные колебания, обусловленные вазоконстрикцией и вазодилатацией мышц сосудов, обусловлены работой активных факторов системы микроциркуляции.

С помощью флаксмоций миогенных колебаний (LFm) предоставляется возможность оценить состояние мышечного тонуса прекапилляров, регулирующего приток крови в нутритивное русло.

С помощью нейрогенных колебаний (LFn) возможно оценивать периферическое сопротивление артериол; увеличение амплитуд нейрогенных колебаний является индикатором снижения сопротивления и возможности усиления кровотока по артериоло-веноулярному шунту при повышении миогенного тонуса [15]

Физиологическая природа нейрогенных колебаний связана с симпатическим адренергическим влиянием на гладкие мышцы артериол и артериолярных участков. Нейрогенная симпатическая активность накладывается на миогенные вазомоции и подчиняет их. При выраженной активации симпатических вазомоторных волокон симпатическая импульсация усиливается, приводя к увеличению нейрогенного компонента артериолярного тонуса, снижению амплитуд осцилляций кровотока в нейрогенном диапазоне. Вышеуказанные колебания кровотока в нейрогенном диапазоне связаны с влиянием тех симпатических волокон, которые иннервируют соответствующие сосуды, т.е. только на локальном участке исследования. Нейрогенная терморегуляторная активность в регуляции микрокровотока значимо зависит от психического состояния человека [19].

Микроциркуляторное русло обеспечивает транскапиллярный обмен O_2 , CO_2 , субстратов, метаболитов, ионов, БАВ. Маркерами стрессовой ситуации являются кортизол, глюкокортикоиды и катехоламины (КА) [17]. Во время стресса и роста концентрации КА в крови снижается среднее значение перфузии, приводящее к росту индекса перфузионной сатурации O_2 в микроциркуляторном русле и сопровождается увеличением оксигенации смешанной крови. Кроме того, падает индекс удельного потребления кислорода и объема перфузии в ткани. Данные изменения связаны со спазмом микрососудов и шунтированием крови ввиду повышения уровня катехоламинов крови [22]. Дилатация и констрикция микрососудов обеспечивается посредством миогенного механизма, который, в свою очередь, зависит от концентрации цитозольного кальция в гладких миоцитах. Концентрация в гладкомышечных клетках меняется при действии стресса, вследствие чего возникает вазоконстрикция или вазодилатация и увеличение или уменьшение перфузии [16]. Стресс развивается по типу симпато-адреналовой реакции. Нейрогенные колебания связаны с симпатическими адренергическими влияниями на гладкие мышцы артериол, что приводит к увеличению активности симпатических вазомоторов, следовательно происходит сужение сосудов. Нейрогенные колебания способны влиять компенсаторно на мускулатуру и кровеносные сосуды, что также может отразиться на колебаниях пульсовой и дыхательной волны. Следовательно, с диагностической точки зрения актуальным является изучение не только амплитуд колебаний в нейрогенном, эндотелиальном и миогенном диапазоне, но и пассивных факторов регуляции кровотока.

Таким образом, выявление стресса объективными методами – актуальная проблема здравоохранения. Предпринимаются попытки осуществить диагностику психологического стресса, используя различные физиологические сенсоры, в том числе ЭКГ, ЭЭГ, фотоплетизмографию [7,13]. Несмотря на полученные результаты, поиску достоверного метода диагностики стресса по-прежнему уделяется много внимания.

Психологический стресс, который опосредуется в основном симпатической нервной системой [16], может стать причиной изменений и кожного кровотока, модулируя нейрогенные осцилляции перфузии. Имеются данные о влиянии стресса на вазомоции и влиянии психогенного фактора на развитие стоматологических заболеваний, успех выздоровления, в частности геронтостоматологических пациентов [22]. Однако по сей день отсутствуют данные о воздействии стресса на развитие осложнений после имплантации, что диктует необходимость проведения дальнейшего исследования.

На основании данных, полученных с помощью ЛДФ-метрии, возможно проанализировать состояния и расстройства системы микроциркуляции, обнаружить различные заболевания на более ранних стадиях их развития, создать базу для более глубокого понимания патогенеза возникающих расстройств микроциркуляции и осуществлять объективный контроль за проводимыми лечебными мероприятиями и индивидуальным подбором фармакологических средств.

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SUMMARY

STRESS AS A PREDICTOR OF PERI-IMPLANTITIS DEVELOPMENT (REWIEV)

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In the era of modern technology is becoming increasingly popular implantation. This method is one of the leading methods in prosthetics of dental patients. Implantation solves a number of problems both aesthetic and functional. However, the percentage of complications, such as peri-implantitis and mucositis, leading to a violation of the chewing function of the dentition, is steadily increasing. To date, the etiology and pathogenesis of post-implantation complications have not been definitively clarified. One of the leading predictors is psychoemotional stress. On the basis of the literature analysis method to study stress, its impact on the development of periimplantitis, is laser Dop-

pler flowmetry, allows you to quickly assess the state of microcirculation of the oral cavity at the stage of diagnosis, prior to the commencement of pharmacotherapy and in the dynamics of treatment and to adjust treatment regimen to prevent postoperative complications. The obtained LDF-grams allow to reflect the state of the regulatory systems of the body, and therefore it is possible to use LDF for the diagnosis of psychological stress.

Keywords: implantation, peri-implantitis, mucositis, diagnostics, stress, laser Doppler flowmetry.

РЕЗЮМЕ

СТРЕСС КАК ФАКТОР-ПРЕДИКТОР РАЗВИТИЯ ПЕРИИМПЛАНТИТА (ОБЗОР)

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В эпоху современных технологий все большую популярность приобретает имплантация, который является одним из ведущих методов при протезировании стоматологических больных. Имплантация решает ряд проблем как эстетических, так и функциональных. Однако, неуклонно растет процент осложнений, таких как периимплантиты и мукозиты, приводящие к нарушению жевательной функции зубного ряда. По сей день окончательно не выяснены вопросы этиологии и патогенеза послеимплантационных осложнений. Одним из ведущих факторов-предикторов их развития является психоэмоциональный стресс. На основании анализа литературы возможным методом исследования стресса, его влияния на развитие периимплантитов является лазерная доплеровская флоуметрия (ЛДФ), позволяющая оперативно оценить состояние системы микроциркуляции полости рта на этапе постановки диагноза, до начала проведения фармакотерапии и в динамике лечения и скорректировать схему лечения с целью профилактики развития послеоперационных осложнений. Полученные ЛДФ-граммы позволяют оценить состояние регуляторных систем организма. Авторы рекомендуют использовать ЛДФ для диагностики психологического стресса.

რეზიუმე

სტრესი, როგორც ფაქტორი-პრედიქტორი პერიიმპლანტიტის განვითარებისათვის (მიმოხილვა)

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თანამედროვე ტექნოლოგიების ეპოქაში იმპლანტაცია სულ უფრო პოპულარული ხდება. ეს მეთოდი ერთ-ერთი წამყვანია სტომატოლოგიური პაციენტების პროთეზირებისათვის. იმპლანტაცია იძლევა როგორც ესთეტიური, ისე ფუნქციური პრობლემების გადაწყვეტის საშუალებას. მაგრამ, განუხრელად იზრდება ისეთი გართულებების სიხშირე, როგორიცაა

პერიმპლანტი და მუკოზიტი, რაც იწვევს კბილთა რიგის ღეჭვითი ფუნქციის დარღვევას. სადღეისოდ საბოლოოდ არ არის დადგენილი იმპლანტაციის შემდგომი გართულებების ეტიოლოგია და პათოგენეზი. ერთ-ერთ წამყვან ფაქტორ-პრედიქტორს წარმოადგენს სტრესი. ლიტერატურის ანალიზის საფუძველზე სტრესის და პერიმპლანტიტების განვითარებაზე მისი გავლენის შესწავლის შესაძლო მეთოდია ლაზერული დოპლეროგრაფიული ფლუორომეტრია, რომელიც პირის ღრუს მიკროციკულაციის სისტემის მდგომარეობის ოპერატიულად შეფასების საშუალებას

ბას იძლევა დიაგნოსტიკის ეტაპზე. ფარმაკოთერაპიის დაწყებამდე და მკურნალობის დინამიკაში, ასევე, ინფორმაციულია მკურნალობის სქემის კორექციისათვის ოპერაციის შემდგომი გართულებების პრევენციის მიზნით.

ლაზერული დოპლეროგრაფიული ფლუორომეტროგრამები იძლევა ორგანიზმის სარეგულაციო მექანიზმების შეფასების საშუალებას, რის გამოც ლაზერული დოპლეროგრაფიული ფლუორომეტრიის ჩატარება მიზანშეწონილია ფსიქოლოგიური სტრესის დიაგნოსტიკისათვის.

PERCEPTION OF ORAL PROBLEMS IN PATIENTS WITH ADVANCED CANCER

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The end-of-life is an essential time period correct perception of which substantially stimulates the process of self-acknowledgment, realisation of self-essence, one’s place and role in the world [15,16]. For every person, as a member of the community, the end-of-life for whom is still far, observation of the other person’s end-of-life and correct understanding of the actions taken during this period contribute to better preparation for the own end-of-life (the role of church and creative art plays particular role here). Moreover, community is obliged to create the environment (ethical and institutional) that helps a person have an honorable ending of life, ensures consideration the importance of maximum respect of one’s faith and wishes. One of the spheres of public activities that is explicitly responsible for this is healthcare that appears to be the institutionally (community) organized relations among the medicine and all other related phenomena (activity, process). In short, healthcare is medicine in the governmental (community) action [7].

Every special period of life, coming out from its medical-biological problems, complies with the concrete direction (field) of medicine. The beginning of life is taken care by “Midwifery” and “Neonatology”, childhood age by “Pediatrics” while the life of elderly people by- „Geriatrics“. This logic suggests that the end of life also must have its medical direction. Until the 2 half of the 20th century no medical care for the end-of-life period existed. From the mentioned period on, medical direction called “Palliative Care” has been developing rapidly [4,12].

Based on the Georgian Laws on „Health Protection“ and „Medical Activities“ palliative care is: “the service via multidisciplinary approach that, through early detection of pain and other physical, psychosocial, mental problems and their correct evaluation as well as treatment, moral support, prevention and relief of suffer, improves the quality of life of a patient, with chronic or/and life threatening diseases, and his/her family members”.

For the last 2 decades Georgia has been taking significant measures that ensured development of a certain system of provision of palliative care for the advanced cancer patients, operation of which is the responsibility delegated among the state, professional organizations and educational institutions.

At the same time the current model needs to be developed further; this requires research besides other activities. Moreover,

in accordance with the definition of the palliative care, the patients with chronic or/and life threatening diseases include the patients of all ages with already advanced chronic incurable diseases as well as the aging people with incurable diseases what for these people is their age [11]. This philosophy was perfectly stated by Shota Rustaveli, Georgian poet of the 12th century: “I passed the years and suffer from my age, the illness worse than others ever” [14].

It must be noted that, in Georgia where elderly people make the fourth part of the population, looking after the aged people is traditionally considered to be the responsibility of the society; this is the issue of worth for family and the nation as whole [8].

International practice proves that the elaboration of the scientifically justified national model of the palliative care for elderly people depends on a certain number of demographic, social and health realities including the aspects such as distribution of the elderly population within the regions of the country, the structure of diseases, economic conditions and social insurance, providing medical support (insurance and targeted state programs) and legislative (legal) service [9,17,19].

Moreover, it is clearly stated that while elaboration of the palliative care model for the elderly as well as incurable cancer patients, the requirements and needs of the given contingent must be considered together with the national-traditional-religious specifics as well as their attitude towards meeting these requirements, with particular focus on the relations (communication) with them at the end of their lives [1,9]; this was proved by our previous research works too [2].

Earlier we carried out a crosssection survey during which the acknowledgment of the end of life and the attitude to it in the cancer patients with the limited life period and the aging people have been reflected and compared through semi-structured format like interview recording and analysis.

As it was revealed the end-of-life needs, perceptions and attitudes are mainly similar in people independently from what causes this sensation of the limitation, be it an incurable disease or the age; the stories of patients with advanced cancer diagnosis as well as the persons over 85 clearly reveal that the complaints and symptoms are overwhelmed by the stories associated with the need of social relations, love and respect as in Abraham Maslow’s hierarchy of human needs and requirements [3].

Besides, while carrying out the mentioned research the problems connected with oral cavity were mentioned only in single cases. It was strangely unexpected as, on the one hand, oral cavity as well as dental problems appear to be common in old people, while, on the other hand, chemotherapeutic treatment in oncological incurable patients also not seldom appears to be the reason of complains on the oral cavity.

We assume that the semi structured interviews that we recorded did not allow to highlight separate symptoms/complaints (including the symptoms/complaints about oral cavity). In other case we must assume that the main problem for the interviewees-acknowledgment of the end-of-life due to the age or illness-overwhelmed all their daily problems so that both elderly patients and patients subject to palliative care had to reveal the demand on free accomplishment of physiological needs, in reality demonstrated intense need of love, relationship, respect to the extent that they paid less attention to the symptoms of disease. In other words we witnessed what Galaktion Tabidze, Georgian poet, said in one of his poems:

„... so the closeness of death changes
the roses and waterfalls in the tunes of the dying swan” [18],

Table 1. First part of the mentioned questionnaire

1. Have you been experiencing any changes in your life after detection of the disease or after treatment?			
a. no	b. slight	c. moderate	d. severe
2. Have you been experiencing any changes in your mouth after detection of the disease or after treatment?			
a. no	b. slight	c. moderate	d. severe
3. Do you have dry mouth?			
a. no	b. slight	c. moderate	d. severe
4. Do you feel any mouth discomfort?			
a. no	b. slight	c. moderate	d. severe
5. Do you have any taste disturbances?			
a. no	b. slight	c. moderate	d. severe
6. Do you have difficulties while chewing?			
a. no	b. slight	c. moderate	d. severe
7. Do you have difficulties while swallowing?			
a. no	b. slight	c. moderate	d. severe
8. Do you have difficulties while speaking?			
a. no	b. slight	c. moderate	d. severe
9. Do you feel sore taste in mouth?			
a. no	b. slight	c. moderate	d. severe
10. Do you suffer from nausea and vomiting?			
a. no	b. slight	c. moderate	d. severe
11. Do you have a burning mouth?			
a. no	b. slight	c. moderate	d. severe
12. Do you use dentures?			
a. yes		b. no	
13. How do dentures fit you?			
a. no		b. yes	
14. Do you have rushing mouth?			
a. no	b. slight	c. moderate	d. severe
15. Do you feel any pain in your mouth?			
a. no	b. slight	c. moderate	d. severe
16. How would you grade your mouth related problems vis-à-vis your general problems? 0.....10..... 20.....30.....40.....50.....60.....70.....80.....90.....100			

while Ilia Chavchavadze, a writer, stated: „when the Sun goes down, stars disappear”. In our case the issue must be understood in the following way: “when a big trouble is approaching us, minor things are no more cause of unrest!” if we deal with this phenomenon, we have to admit that elderly people like incurable cancer patients who can feel some limitation of the life left, appear to be very interesting subjects for the psycho-medical research [10,13].

We aimed to verify if the less attention from the sides of patients towards the oral cavity symptoms/complaints were caused by the “poor” format of semi-structured interviews that were not focused on the oral status.

With the purpose to meet the presented objective, we decided to address the below given three tasks:

- To find out to what extent the oral cavity problems cause complaints in patients with incurable cancer versus their general complaints;
- What is the compliance between the complaints of the patient with incurable cancer and the reality stated and assessed by the dentist;

- How manageable the pathologies of oral cavity are for the patients with advanced cancer.

Material and methods. 50 patients, aged 20 -75, with advanced cancer were involved in the study; the patients were provided integrated palliative treatment (care) in the Cancer Centre (in the Department of Chemotherapeutic and Palliative Care of the Batumi High Technology Hospital Clinic “Medcentre”) in the Autonomous Republic of Ajara from 10 January 2017 until 30 November 2018.

Criteria for the involvement in the research:

1. Verified advanced cancer;
2. The sample involved in the survey had to speak Georgian language at the satisfactory level;
3. Cognitive skills of those involved in the survey had to be adequate (they have to be able to understand the questions they are asked and respond thoughtfully).

Adequate cognitive function (was confirmed by the results of Mini-mental State Examination investigation provided by their family doctors before their admission to Cancer Center in the

Batumi High Technology Hospital Clinic «Medcentre».

Criteria of exclusion from the research:

- 1.No or low competences of Georgian language;
- 2.Inadequate cognitive skills.

For the research we used a questionnaire the part of which was answered by the people involved in the research who had simply to circle one out of the proposed alternatives. The below presented table (Table 1) represents the first part of the mentioned questionnaire.

After filling the questionnaire we recorded the additional information related to the “problems with oral cavity” provided with the patients via in-depth interview. The recorded information was processed then and together with the from the questionnaire was the subject of the qualitative and quantitative analysis.

The second part of the questionnaire was filled by a researcher/dentist on the ground of the information obtained through the data in the questionnaire and the examination of oral cavity of the patient (Table 2).

Table 2. Second part of the questionnaire
Second Part of the Questionnaire

Date: _____

1.Name, Surname: _____

2. ID Number: _____

3.Age: _____

4.Address: _____

5.Telephone: _____

6.Profession and Occupation: _____

8. Previous and accompanying diseases: allergy, pediatric infectious diseases, Cardio-vascular disorders, Respiratory system disorders, Digestive system disorders, Urinary system disorders, Nervous system disorders, Endocrine system disorders;

Note: _____

9.Primary diagnosis: _____

10.History of illness: _____

11. Passed therapy: chemotherapy, radiotherapy and surgery; _____

12. Current therapy: chemotherapy, radiotherapy and surgery; _____

13.Planned therapy: chemotherapy, radiotherapy and surgery; _____

14.Oral cavity anamnesis: (how do the existing oral cavity complaints disturb the patient and to what extend do they decrease quality of their life) ;

-dry mouth: _____

-mouth discomfort: _____

-taste disturbances: _____

-difficulty chewing and swallowing: _____

-difficulty speaking: _____

-mouth sores: _____

-mucus layer diseases (candidosis, stomatitis, etc.); _____

Notes: _____

15.External examination:

Face skin color: _____

Symmetry: _____

Tumors: _____

Regional lymph nodes: enlarged, painful, movable; _____

Lips and mouth angles state: norm, splits; _____

Mucosa state: tongue, cheeks and palates; _____

16.Occlusion: normal, mesial, distal, edge-to-edge, nonocclusion, crossbite; _____

Note: _____

17. Formula:

8	7	6	5	4	3	2	1	1	2	3	4	5	6	7	8
8	7	6	5	4	3	2	1	1	2	3	4	5	6	7	8

Dentures type: removable full or partial; _____

Dentures fitting: yes, no; _____

Necessity of dentures: yes, no; _____

18.Hygiene state: good, bad; _____

19. How a patient takes care of his/her mouth _____

20.Periodontal diseases: _____

Note: _____

Table 3. The diagnoses of patients (n=50)

Diagnosis	Number	
	Women	Men
Malignant tumor of bronch and lung	2	15
Ductal carcinoma of mammary gland	5	
Prostate adenocarcinoma		3
Malignant ovarian tumor	3	
Malignant tumor of pancreatic head	1	1
Malignant tumor of rectus	1	1
Follicular limphoma	1	1
Malignant tumor of Urinary bladder		2
Malignant cervical tumor of uterus	2	
Malignant lateral side tumor of tongue	1	
Malignant skin melanoma		1
Malignant tumor of thyroid gland	1	
Nonhodgkin limphoma		1
Malignant tumor of connective and soft tissues (myxosarcoma)	1	
Malignant tumor of kidney		1
Kaposi's sarcoma		1
Malignant tumor of stomach		1

The presented questionnaire (both parts) was created based on consultations with international experts in oncology, palliative care and dentistry;

We conducted the pilot survey to evaluate the validity of the questionnaire. In order to check if each question was understandable, for the pilot survey we chose 6 patients with advanced cancer and 6 practically healthy patients. All 12 patients involved in the survey coped with the first part of the questionnaire without complications as well as collaborated with the dentist at the satisfactory level in the process of filling the second part of the questionnaire.

Results and their discussion. Out of the 50 cancer patients with incurable disease involved in the survey 20 were women and 30-men. The diagnoses of all 50 patients are presented in the Table 3.

31 (62%) patients were diagnosed to accompanying disease. Out of those 31 17 (55%) patients had 2 and more accompanying diseases.

Out of 50, 33 had undergone various treatments. 21 patients had undergone complex treatment: 3 patients had been operated; they also had radiotherapy and chemotherapy. 2 patients had been operated and treated with radiotherapy; 9 patients had been operated and treated with chemotherapy, while 7 patients had been administered chemotherapy and radiotherapy.

While being involved in the survey these 33 patients were not the subjects to cancer treatment in the radical or palliative regime; they were staying in hospital or at home under symptomatic treatment and palliative care.

17 patients were diagnosed late, at the 4th stage of the disease. Due to severeness of the disease they were rejected from radical treatment methods and transferred to the 4th clinical group. This group of patients directly became the subjects of palliative care.

Oral cavity appeared to be the cancer localization area for one patient only.

Based on the data of each part of the questionnaire for the patients we prepared 2 tables where the data were grouped in

accordance with the gender and 3 aging groups of the patients (20-44; 45-64; 65 and older) (Tables 4 and 5).

Slight and severe changes in the quality of life were stated by 42 (84%) patients including 26 men and 16 women. Out of these 42 patients 24 were 65 years old and more. Cancer was expected to have changed the quality of life of young people more (due to disease, everyday routine must have been changed for them more [worsen]). It is possible that the change in the quality of life in the elderly people is the combination of worsening the life due to their disease and the age and, therefore, it is perceived more painfully.

Mild and excessive nausea and/or vomiting were stated by 15 (30%) out of 50 patients out of which 7 were women and 8- men. 8 out of those 15 patients were 65 and older. The stated two symptoms are related to cancer (or they appear to be the side effects of treatment) [6]. It has to be underlined that all patients with these symptoms reported the decrease of the quality of life.

Out of 50 patients, involved in the survey, 21 (42%) wore dentures; 18 out of those 21 patients were 65 and older. 6 (29%) out of 21 patients expressed dissatisfaction with dentures. Comparison of given data with the data on the change of the life quality confirms that dissatisfaction with dentures should not be the determinant of the life quality evaluation.

Changes in oral cavity were stated by 30 out of 50 patients including 13 women and 17 men. 19 (7 women and 12 men) out of these 30 patients were 65 and older. It is interesting that sensation of changes in oral cavity were rarely connected with "bad work" of dentures.

All patients revealed having at least one symptom out of the 10 symptoms of oral cavity listed in the questionnaire (Fig.). Mild and excessive symptoms, in six groups selected in accordance with gender and age, range between 2 and 23 %, while the absence or minor representation of complaint ranges between 2-52%.

What is more, the absence of symptoms or "minor discomfort" in oral cavity were mainly stated (30-52%) by 65 year old and older patients. At the same time, this data stays same statistically in women and men.

Table 4. Symptoms in Cancer Patients (as seen by the patients)

Gender	Symptoms			Changes in life	Changes in oral cavity	Dry mouth	Mouth discomfort	Taste disturbance	Difficulty swallowing	Difficulty speaking	Mouth sores	Nausea and vomiting	Burning mouth	Dentures	Satisfaction with dentures	Mouth rashes	Mouth pain	Grading of oral cavity complaints vis a vis general complaints (%)																	
	no	slight	severe																																
Female	20-44	1	2	1	1	1	1	1	1	1	3	1	2	1	1	3	1	1																	
	45-64	2	1	2	1	1	1	1	1	1	4	2	1	3	2	1	5	2	1																
	65 >	1	5	7	5	3	4	3	3	3	1	9	3	10	2	2	10	2	1																
Male	20-44	1	2	1	2	1	2	1	2	1	1	1	1	1	3	3	3	1	1																
	45-64	1	8	4	1	3	2	2	4	1	1	2	4	3	7	2	8	1	7																
	65 >	2	2	4	10	12	5	1	3	3	1	10	4	1	15	1	1	1	1																
Sum	5	3	13	29	30	18	2	13	7	17	35	9	5	23	29	15	6	44	3	4	2	43	3	1	3	27	2	5	2	4	1	1	2	1	1

Table 5. Data Obtained from the Questionnaire and Oral Cavity Examination of Cancer Patients

Gender	Indicators	Cancers diseases	Accompanying disease	Passed therapy	Current therapy	Planned therapy	Facial colour	Face symmetry	Tumours on face	Regional lymph nodes	Lips and mouth angles	Occlusion	Number of teeth	Types of dentures	Necessity of dentures	Hygiene state	Periodontia (Biomax)																							
																		Cytotoxic drugs	Respiratory disorders	Digestive system disorders	Uterus system disorders	endocrine system disorders	Surgery	Chemotherapy	Radiotherapy	Normal	Asymmetric	Yes	Enlarged	Pathly	Movable	Normal	Slight	No	Normal	Distal	Noncrossbite	Crossbite	Superoclusion	Full dentition
Female	20-44	1	1	2	2	1	3	3	3	3	2	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1		
	45-64	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	65 >	4	2	5	11	9	11	7	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12
Male	20-44	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	45-64	3	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	65 >	1	9	4	2	1	4	6	5	6	1	18	13	5	7	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Sum	3	18	9	17	4	5	11	14	13	21	19	3	24	3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	

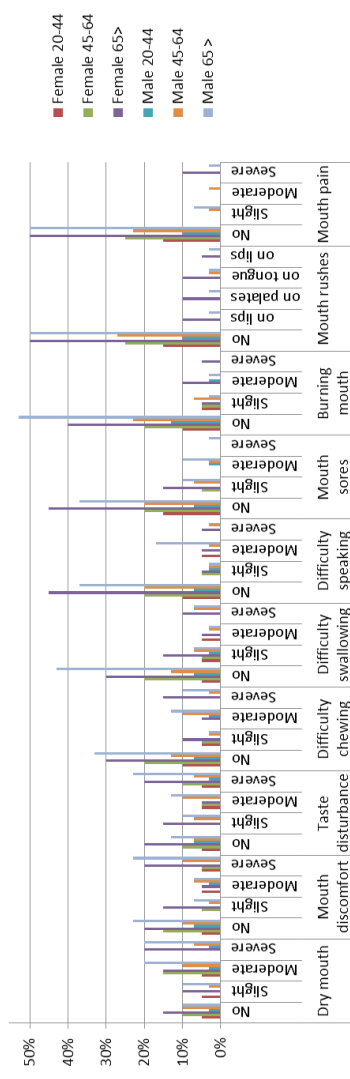


Fig. Frequency of Oral Cavity Problems in Accordance with Gender and Age

Only one woman, diagnosed to tongue cancer, stated that oral cavity was her main complaint. All the above presented confirms that incurable cancer patients do not “consider” oral cavity complaints as a significant problem.

Examination of the patient’s mouth by the researcher (dentist) revealed splits on lips and mouth corners in 13 (26%) patients including 5 (10%) women and 8 (16%) men. Splits or cheilitis can be explained with the dryness in mouth or the lack of saliva that, in its side, is caused due to hypofunction of salivary glands. It must be underlined that 6 out of 13 patients did not complain about oral cavity at all.

Occlusion or physiological (normal) relevance of the rows of low and upper teeth [5] is changed in the case of 50%; that in the majority of the patients is due to deficiency of teeth and absence of dentures. When dentures are not applied on time after missing teeth due to various reasons, low and upper teeth start mismatching that might be followed by pathological changes in temporomandibular joint too [5]. The presented table demonstrates that not a single patient had full dentition. Moreover, out of the group of elderly people- 5 (10%) women and 9 (18%) men - had adentia (complete lack of teeth); 13 out of 14 patients wore dentures. In 7 cases dentures were of a bad quality suggesting the need of other dentures. The rest 36 patients were identified to have missing teeth to various amounts. Out of 50 patients only 25 (50%) wore dentures, while 16 (32%) needed to be provided with them urgently, among them 7 patients with adentia. 34% of men and 14% of women were identified to have various types of dental plaques. Condition of oral cavity in correlation with disregard of oral cavity hygiene. Diseases of periodont have been identified too the frequency which is increasing in parallel of getting older.

The above stated proves that the sample needs to improve the oral cavity hygiene. Moreover, if we consider that the presented condition could be unlikely connected to cancer or its side effects, we can conclude that the problems of oral cavity are more of common/general nature.

Adaptation to the lack of teeth and even more, full adentia, when the people involved in the research chose “no” or “slight” out of the alternative answers focused on the oral cavity problems, while filling the questionnaire, reveals a lack of education and awareness on the the oral cavity in general and in particular, the physiology and hygiene of teeth.

Conclusions. 1. Based on the above stated we must assume that the semi-structured interview format (without special focus on the complaints on the oral cavity) “does not appear guilty” in reporting oral cavity complaints/problems as insignificant by the patients. Cancer patients consider these problems as less important;

2. Conditions recorded by a dentist are more severe and serious in comparison to what the patients stated;

3. Furthermore, it must be taken into consideration that the conditions and pathologies of oral cavity registered during our research are subject to improvement and treatment and, relevantly, their correct management has potential to improve the quality of life for incurable cancer patients.

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SUMMARY

PERCEPTION OF ORAL PROBLEMS IN PATIENTS WITH ADVANCED CANCER

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The results of the previous research, aimed to study the perception of and attitude to the end-of-life in the incurable cancer

patients as well as the elderly aged 85 and older, revealed that independently from the cause of the life limitation, perceptions of and attitudes to the end-of-life are mainly similar. Moreover, it must be highlighted that the complaints on oral cavity were stated only by the single patients from the sample.

We aimed to verify if the less attention from the sides of patients towards the oral cavity symptoms/complaints were caused by the “poor” format of semi-structured interviews that were not focused on the oral status.

The sample of the research included 50 advanced cancer patients aged 20 to 75, under integrated palliative treatment (care). We applied for two-part questionnaire in the first part of which the patients had to choose one out of the alternative answers to the concrete questions related to oral cavity problems. The second part of the questionnaire was filled by the researcher/dentist based on the information obtained through examination of the patient’s oral cavity.

Based on the results of the data processing it was concluded that conditions recorded by a dentist were more severe and serious in comparison to what the patients stated.

Furthermore, it must be taken into consideration that the conditions and pathologies of oral cavity registered during our research are subject to improvement and treatment and, relevantly, their correct management has potential to improve the quality of life for incurable cancer patients.

Keywords: incurable cancer, oral cavity, palliative care.

РЕЗЮМЕ

ВОСПРИЯТИЕ ПРОБЛЕМ ПОЛОСТИ РТА У ПАЦИЕНТОВ С ЗАПУЩЕННЫМ РАКОМ

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Целью исследования явилось определение причин невыявления жалоб на патологии ротовой полости и симптомов заболевания в интервью полу-структурированного формата с отсутствием фокусирования на состоянии ротовой полости.

Исследовано 50 онкологических больных в возрасте 20-75 лет, которые получали интегрированное паллиативное лечение.

В процессе исследования использованы анкеты-опросники, состоящие из 2 частей. В первой части исследуемые пациенты отвечали на заданные вопросы по поводу жалоб на состояние ротовой полости путем выбора ответа из нескольких предложенных вариантов.

Вторую часть анкеты заполнял врач-стоматолог после осмотра ротовой полости исследуемых пациентов.

В результате анализа полученных данных установлено, что состояние, зафиксированное врачом-стоматологом, более тяжелое и серьезное, чем в жалобах пациентов.

На основании результатов обработки данных проведенного исследования авторами делается вывод, что состояние, зарегистрированное стоматологом, было более тяжелым и серьезным в сравнении с жалобами пациентов.

Следует учитывать, что состояние и патология полости рта, выявленные в ходе проведенного исследования, подлежат улучшению и лечению. Таким образом, их правильное ведение может улучшить качество жизни больных тяжёлыми формами рака.

რეზიუმე

პირის ღრუსმხრივი ჩივილების აღქმა კიბოს შორს-წასული ფორმებით დაავადებულ პაციენტებში

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აგტორების მიერ ადრე ჩატარებულია სიცოცხლის შეზღუდული ხანგრძლივობის გამაცნობიერებელი ონკონკურაბელური პაციენტების და 85 და მეტი წლის მოხუცთა ჯგუფების მიერ სიცოცხლის მიწურულის აღქმისა და დამოკიდებულებების ანკეტური კვლევა. დადგენილია, რომ სიცოცხლის ლიმიტურობის განმარტობის გარემოებების მიუხედავად, სი ცოცხლის მიწურულის აღქმა და დამოკიდებულებები მეტწილად მსგავსია. ამასთანავე, აღსანიშნავია, რომ გამოკვლეულ კონტინენტში მხოლოდ ერთეულებმა აღნიშნეს პირის ღრუსმხრივი ჩივილები.

წინამდებარე კვლევის მიზანს წარმოადგენდა ნახევრად-სტრუქტურირებული ინტერვიუს ფორმით გამოკითხვის მეთოდით პაციენტების მხრივ პირის ღრუს სიმპტომებზე/ჩივილებზე ყურადღების გამახვილების/არგამახვილების მნიშვნელობის შეფასება.

გამოკვლეულია 20-75 წლის ასაკის 50 ონკონკურაბელური პაციენტი, რომლებიც იღებდნენ ინტეგრირებულ პალიატიურ მკურნალობას (მზრუნველობას). გამოყენებულია ორნაწილიანი ანკეტა-კითხვარი. პირველ ნაწილში დასმულ კონკრეტულ შეკითხვებს პირის ღრუსმხრივი ჩივილების თაობაზე კვლევაში ჩართული პირები უპასუხეს შეთავაზებული კერსიებიდან ერთ-ერთის უბრალო შემოსახვის გზით; ანკეტის მეორე ნაწილს, კვლევაში ჩართული პირების პირის ღრუს დათვალეიერების შედეგად მიღებული ინფორმაციის საფუძველზე, ავსებდა მკვლევარი (ექიმი-სტომატოლოგი).

მიღებული მონაცემების დამუშავების შედეგად დადგინდა, რომ ექიმი-სტომატოლოგის მიერ დაფიქსირებული მდგომარეობა უფრო მძიმე და სერიოზულია, ვიდრე ეს ასახულია პაციენტების სუბიექტურ აღქმებსა და ჩივილებში. ეს მიუთითებს, რომ ადრე ჩატარებული კვლევის შედეგები არაა პირობადებულად გამოყენებული ანკეტა-კითხვარის სტრუქტურის ხარვეზით პირის ღრუსმხრივი ჩივილებზე არასათანადო აქცენტის თვალსაზრისით.

გასათვალისწინებელია, რომ პირის ღრუს ის მდგომარეობები და პათოლოგიები, რაც მკვლევარის მიერ იქნა აღწერილი, ექვემდებარება მართვას (გაუმჯობესებას და მკურნალობას), რაც, შესაბამისად, გულისხმობს ონკონკურაბელურ პაციენტთა ცხოვრების ხარისხის გაუმჯობესებას.

MODERN TREATMENT METHODS OF PHLEGMON IN THE MAXILLO-FACIAL AREA AND NECK

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The lymphatic system enters the human blood system and is actively involved in ensuring the immune response of the body. It duplicates the vasculature, consisting from lymphatic capillaries, blood vessels, and evenly distributed lymphoid tissue, which is a part of the structure of the lymph nodes and group lymphatic follicles of the small intestine (Peyer's patches) and others. According to the evidence of the world's lymphologists, the lymphatic system is the main organ of the human immune system, most of the lymphocytes are located in it [3,4,11]. The lymphatic system is involved in all pathological processes in the macroorganism [2,5,7] since the division and further adherence of bacteria and toxins occurs through the lymphatic vessels and lymph nodes and creates so-called accumulation of toxins, therefore the endolymphatic injection of antibacterial agents of immunocorrective is pathogenetically justified [1,9]. Antibacterial saturation by the lymphotropic way creates high concentrations of anti-inflammatory and other drugs on the path of movement of microorganisms and bacteroids, contributing to a rapid immune response and movement of antibacterial drugs to meet microorganisms.

One of the many distinguishing features of odontogenic infection is that the patient's body cannot cope on its own, without remedial measures, to stop the flow of microorganisms from the source of infection. And it means it is useless to hope on the self-healing and the complete elimination of the infectious focus. At best, the process is chronicized, a center of odontogenic infection is in a state of equilibrium with the patient's body [6,8,10].

Objective: to increase the effectiveness of treatment of phlegmon of the maxillofacial area and neck with the influence on pathogenic factors, complementing the main treatment plan with the injection of the second antibiotic in a regional lymphatic way.

Material and methods. All procedures carried out in the study with the participation of patients corresponded to the ethical standards of the institutional and national research committee, as well as the Helsinki Declaration (1975) and its revision, 1983. We have carried out the diagnostics and complex treatment of 100 patients with odontogenic phlegmons who were hospitalized in the department of maxillofacial surgery at the Department of Surgical and Therapeutic Dentistry of the State Establishment "Zaporizhzhya Medical Academy of Postgraduate Education of the Ministry of Health of Ukraine" in City Clinical Hospital for Emergency and Medical Care, Zaporizhzhya during 2016-2017. Patients were divided into 2 groups: 1 gr - 45 patients were treated with intramuscular injection of "ceftriaxone". 2 gr - 55 patients whose treatment consisted of both the traditional method and the lymphatic injection of "Lincomycin" (antibiotic).

All patients got a complete examination upon admission to the hospital. Surgery was performed according to the generally accepted technique under the local or general anesthesia depending on the prevalence of the inflammatory process and it deals with a wide dissection and drainage of the purulent focus.

Patients received common antibacterial and anti-inflammatory therapy; the correction of water and electrolyte balance was carried out.



Fig. 1. Inflammatory process targeting

From the very first hospital stay, all hospitalized patients received antibacterial therapy, regardless of the location and extent of the infectious-inflammatory process. Choosing places for lymphotropic injection of antibacterial drugs, we proceeded from the regional principle on the basis of available literature data about the structure of the lymphatic system of the maxillofacial area, taking into account the anatomical and physiological principle of lymphatic drainage.

For directional exposure on the inflammatory process in the lower jaw body, as well as adjacent sublingual, submandibular, pterygomaxillary and chewing cellular tissue spaces, the antibacterial drug was injected strictly hypodermically 1 cm below and laterally to the mastoid of the temporal bone on the side of inflammation.

In the submental area, drugs were injected into submucosally in the area of the mandibular nerve exit from the lower jaw on both sides using the subperiosteal anesthesia method. We have injected lymphotropic antibacterial drugs intracutaneously in the nasolabial fold on the side of inflammation to influence on the infectious inflammatory process in the upper jaw, upper lip, infraorbital, zygomatic, cheek areas.

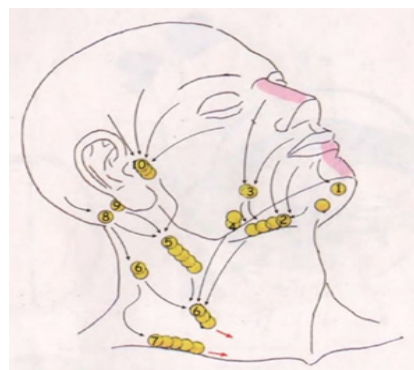


Fig. 2. The location of the lymph nodes of the maxillofacial region

With the spread of the inflammatory process in the parotid-masticatory area or the parenchyma of the parotid salivary gland, the

Table 1. The distribution of patients with phlegmons of the maxillofacial area and neck depending on the age and sex corresponds

Sex	Age (years)				Total
	15-20	21-40	41-60	61 and older	
Men	6	43	9	2	60
Women	7	22	10	1	40

Table 2. Indicators of the general blood test, which were taken upon admission to the hospital, convincingly show the development of inflammatory processes in patients

Location area	Number
Chin	4
Submandibular	35
Mandibular	2
Cheekbone	2
Wing maxillary	12
Parotid-chewing	10
Pharyngeal	15
Infraorbital	5
Neck	4
Floor of the mouth	10
TOTAL	100

lymphotropic antibiotic drugs were injected intracutaneously under the base of the ear tragus. It is possible to do the simultaneous injection of an antibacterial drug from several points based on the severity of inflammatory process (Fig. 2). The course of lymphotropic antibiotic therapy lasted 5-7-10 days.

For the treatment of patients with phlegmon of the maxillofacial area and neck, regionally antibacterial lymphotropic therapy was used as an additional way of injecting drugs to the basic principle of treatment. Ceftriaxone, an antibiotic of the 3rd generation of cephalosporins group, was chosen as the main antibacterial drug. In addition, Lincomycin 30%, an antimicrobial medicine of the lincosamide group, was injected lymphotropically as a second antibacterial drug.

The distribution of patients was carried out taking into account sex and age, as it is shown Table 1. The most common hospital patients - are people of working age, from 20 to 40 years (90.9%). All 100 patients included in the comparison group of the etiological factor of the purulent-inflammatory process of the maxillofacial area the teeth were affected by complicated caries.

In particular, from the topographic classification of phlegmon of the maxillofacial area, a purulent-inflammatory process can spread to one, two and more areas. The distribution of inflammatory process by anatomical cellular spaces corresponds to the Table 2.

According to the Table 2, purulent-inflammatory diseases of the maxillofacial area are often localized in the submandibular, parotid-chewing and pharyngeal areas and the floor of the mouth. In 63 patients, the spread of acute purulent-inflammatory process was observed in two or more anatomical cellular spaces.

At the time of hospitalization of various indicators of white blood hemogram any comparison was not observed between the groups. In 86 (86%) patients, red blood cells did not exceed normal values and only in 14 (14%) patients their value

was $4 \times 10^{12}/l$. Hemoglobin in 24 (24%) patients was less than 120 g/l, while the remaining 76 (76%) patients were within the normal range. Changes in leukogram values were different. In 35 (35%) patients, the number of leukocytes was within the normal range, in 7 (7%) patients leukopenia was observed less than $4 \times 10^9/l$ and only in 58 (58%) patients leukocytosis more than $11 \times 10^9/l$ was observed according to the analysis. An increase in the number of rod neutrophils to 7-8%, and segmented neutrophils accounted for up to 70%, also was noticed only in 45 (45.5%) patients. At the same time, there was a decrease as for lymphocyte content in all patients: severe lymphopenia (5-15%) in 41 (40.5%) patients, in 14 (14.1%) patients a decrease in their number (to 20-30%) was noticed. In 20 (20.3%) patients, the number of lymphocytes did not pass their lower limit of the rate (from 15 to 20%), and only in 25 (25%) patients the level of lymphocytes was within the physiological limits. A decrease in the number of lymphocytes with a low number of leukocytes proves that this is one of the signs of the torpid course of the disease. Hematological parameters among practically healthy individuals and patients of the 1st and 2nd groups with phlegmon during hospitalization correspond to Table 3.

Thus, 40.5% of hematological signs of impaired immune response took place. It indicates on the development of inflammation which is not favorable for a quick recovery of the patient. The evidence of low clinical activity of the inflammatory process were indicators of ESR, which did not rise 20 mm/hour in 33 (33.5%) patients, in 45 (53.2%) patients their values were in the range of 20-40 mm/hour, and only in 22 (22.2%) patients the indicators were higher than 40 mm / hour of the physiological norm.

Results and their discussion. As a result, it was found that among 17.3% of patients with odontogenic phlegmons of the maxillofacial area a torpid stage of the disease with blurred clinical signs was observed.

Table 3. Hematological parameters among practically healthy individuals and patients of the 1st and 2nd groups with phlegmon during hospitalization

Index	Healthy faces (n=20)	I group (n=45)	II group (n=55)
Red blood cells, (10 ¹² /l)	4,64±0,06	4,31±0,08*	4,27±0,08*
Hemoglobin, (g/l)	141,0±1,7	132,6±2,8*	134,7±2,1*
Color indicator, (c.u.)	0,91±0,01	0,89±0,01	0,89± 0,01
White blood cells, (10 ⁹)	5,88±0,14	11,02±0,4*	12,48±0,4*
Rod neutrophils, (%)	3,05±0,29	8,53 0,74*	9,69±0,61*
Segmented neutrophils, (%)	61,18±0,74	64,48±1,65*	66,09±1,44*
Lymphocytes, (%)	28,09±0,6	18,82±1,4*	16,72±1,2*
Monocytes, (%)	5,45±0,38	6,04±0,4	5,03±0,2
Erythrocyte sedimentation rate mm/h	6,0±0,6	29,2±1,7*	28,2±1,5*

* – significant differences from the group of healthy individuals ($p < 0.05$)

Table 4. Comparative characteristics of clinical indicators in patients with phlegmon of the maxillofacial area and neck

Indicators	I group (n=45)	II group (n=55)
General improvement, (day)	4,7±0,6	2,4±0,7
Pain Relief, (day)	3,2±0,4	2,5±0,8
Reduction of purulent discharge, (da)	6,2±0,7	3,4±0,6*
Appearance of granulations, (day)	8,5±0,9	4,2±0,5*
Wound cleansing, (day)	11,2±0,8	6,2±0,7*
Bed day (day)	15,7±1,2	9,5±0,6*

* - statistically significant differences from the control group ($p < 0.05$)

In 58 (58.3%) patients, accompanying somatic diseases, such as chronic diseases of the digestive system and the bronchopulmonary system, as well, were presented.

Before hospitalization, 57 (57%) patients did not seek any medical help from a doctor, they did self-medicated, used antibacterial drugs without a doctor's prescription. 43 (43%) patients were at the outpatient appointment with a dental surgeon before referral to hospital. They were carried out anti-inflammatory therapy with antibiotics, sulfonamides, analgesics, UHF, compresses. Many patients took these drugs in full dosages and in an incomplete course. The course of the inflammatory process from the first clinical symptoms to hospitalization in the majority of patients (87%) was more than 3 days. In these patients, lymphotropic therapy accelerated the result of the inflammatory process and led to the recovery of the body in shorter periods. The results of the observation showed a positive dynamic as for the treatment of patients of the second group with regionarny injection of lincomycin in addition to the main method of treatment; their length of stay in the hospital decreased, compared with patients of the first group.

On the 4th day, there was noticed a positive dynamic in the results of blood tests (an increase in the number of red blood cells, a decrease of the ESR indicator, a decrease in the number of leukocytes, etc.), a decrease in symptoms of general intoxication of the body and a decrease in local signs of inflammation. The wound was cleansed and granulations appeared in it three days earlier. Normalization of body temperature in patients of the second group took place 3-4 days earlier, in patients of 1st group, a decrease in body temperature was

accompanied by its periodic rises. In the second group of patients simultaneously with the normalization of body temperature, the general condition improved, sleep and appetite returned to normal. In group 1, the same indicators appeared later for 2-4 days. It was also established that with the regionarny injection of the antibiotic Lincomycin, its concentration in the exudation lasted up to 24 hours, and was higher than with the traditional method of antibacterial drug injection.

With the injection of the drug by the regionarny method, it penetrates into the blood from the lymphatic system. Associated it with the fact that the movement of the lymph through the vessels is not large and amounts to 0.4-0.5 m/s, the antibacterial drug entering the blood from the lymphatic system periodically, in small doses, keeps a positive concentration of the antibiotic in the lymphatic system and stay longer in blood. This explains the preservation of a high concentration of the antibiotic in soft tissues in the area of the inflammatory process in comparison with the similar injection by the intramuscular method.

The maximum concentration of Linkomicin in the blood after endolyphatic injection is slightly lower than the concentration of the drug injected intramuscularly. However, if we consider that the maximum intramuscularly injected ceftriaxone concentration occurs on the 2nd hour after application and quickly decreases (by 93 % by 12 hours), while the maximum amount of Linkomicin injected endolyphatically is found in the blood 4 hours later and decreases on the 12th hour, it is only 79.5 %. A day later, regardless of the presence of pathways, lincomycin is not detected in the blood.

Using this method of injection of antibacterial drugs, three types of complications were identified: the formation of infiltrates, skin necrosis, allergic reactions.

The data obtained by us testified about significant changes in the immune status of (17.3%) patients with phlegmon of maxillofacial and neck. In the process of traditional antibacterial therapy in the first group, stabilization of the general condition in 40 (88%) patients was noticed on 4th-5th days after surgery, in 5 (12%) patients - on 4th-5th days after the wound repair and elimination of streaks in hidden checkered spaces. Reducing of the pain occurred on the 5th day. Observing the mild inflammatory reaction and the treatment, there was no evidence as for a restriction of the process in patients of the first group. In 17 (37.7%) patients, the decrease in suppuration occurred on 6th - 7th day, scanty, sluggish granulations were formed in the wound on 8th-9th day. The average number of days in the hospital for patients of the first group was 13.21 ± 1.3 days.

In 5 (12 %) patients, recovery took a longer time, since several interfascial spaces and anatomical areas were involved Table 4. Serious-purulent discharge was maintained on the background of the already formed granulation tissue; up to 6th-7th day soft tissue infiltration along the periphery of the wound did not diverge until 8-9th day. A decrease in the number of suppuration in 4 (8.8%) patients was observed on the 8th day, fine-grained granulations appeared on the 9th day, a complete cleansing of the wound and the convergence of the edges were noted on the 13th day. The average hospital stay of these patients was 15.34 ± 1.1 days, since these patients had an accompanying diagnosis of diabetes. The dynamics of clinical signs in patients of the first group was more marked.

Improvement of the clinical picture and stabilization of the general condition in 53 (96.4 %) patients of the second group was on the third day after surgery, in 43 (95.6 %) patients of the first group - on 5th-6th day, the intensity of the pain syndrome decreased on average on 4th-5th day. In 48 (87.3 %) patients of the second group suppuration was already absent on 2nd-3rd day, the formation of granulations - on 3rd-5th day, and complete cleansing and drawing closer of the wound edges - on 6th-8th day. In 4 patients of the first and second groups, the healing time of the post-operative wound was longer: the cessation of suppuration was noticed by 6th-7th day, the appearance of granulations - by 7th-8th day, complete cleansing and boundary wound closeness - by 10th-12th day.

Conclusions. 1. Regionary lymphatic injection of the antibiotic in the treatment of phlegmon of the maxillofacial area and neck leads to an accelerated appearance of the second phase of the wound process, more rapid detoxification of the body, reducing the number of complications in patients and the prevalence of the marked positive dynamics in the lymphogram.

2. Studies have shown that a low-molecular antibiotic, regionary injected into the lymphatic system, accumulates at higher concentrations in various organs and tissues compared to the main broad-spectrum antibacterial drug when injected intramuscularly.

3. When comparing the results of treatment of patients with localized forms of purulent-inflammatory diseases of the maxillofacial area and neck, the groups showed a reduction in the course of the disease and the patient's recovery at least twice.

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SUMMARY

MODERN TREATMENT METHODS OF PHLEGMON IN THE MAXILLO-FACIAL AREA AND NECK

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Objective of the investigation - to increase the effectiveness of treatment of phlegmon of the maxillo-facial area and neck with the influence onto the pathogenic factors, complementing the main treatment plan with the injection of a second antibiotic in a regionary lymphatic way.

100 patients with acute inflammatory odontogenic diseases of the maxillofacial area were examined. Patients received both traditional medical treatment and regional lymphotropic antibiotic therapy added to the main method of treatment as well.

Regional lymphotropic antibiotic injection in the treatment of phlegmon of the maxillofacial area and neck leads to an accelerated onset of the second phase of the development of the wound process, more rapid detoxification of the body. Key

words: acute inflammatory odontogenic diseases, maxillofacial area, lymphatic system.

Keywords: phlegmon of the maxillofacial area and neck, lymphatic system.

РЕЗЮМЕ

СОВРЕМЕННЫЕ МЕТОДЫ ЛЕЧЕНИЯ ФЛЕГМОН ЧЕЛЮСТНО-ЛИЦЕВОЙ ОБЛАСТИ И ШЕИ

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Лимфатическая система – входит в единую кровеносную систему человека и активно участвует в обеспечении иммунного ответа организма.

Цель исследования - повысить эффективность лечения флегмон челюстно-лицевой области и шеи влиянием на патогенические факторы, дополняя основной план лечения введением второго антибиотика регионарным лимфатическим путем.

Обследовано 100 пациентов с острыми воспалительными

одонтогенными заболеваниями челюстно-лицевой области. Больные получали как традиционное медикаментозное лечение, так и к основному методу лечения включали регионарную лимфотропно антибактериальную терапию. Заключение. Регионарное лимфатропное введение антибиотика при лечении флегмон челюстно-лицевой области и шеи приводит к ускоренному наступлению второй фазы развития раневого процесса, более быстрой дезинтоксикации организма.

რეზიუმე

ყბა-სახის და კისრის მიდამოების ფლეგმონების მკურნალობის თანამედროვე მეთოდები

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კვლევის მიზანს წარმოადგენდა ყბა-სახის და კისრის მიდამოების ფლეგმონების მკურნალობის ეფექტურობის გაზრდა პათოგენურ ფაქტორებზე ზემოქმედებით, მკურნალობის ძირითად სქემაში რეგიონული ლიმფური გზით მეორე ანტიბიოტიკის დამატებითი შემოყვანით.

გამოკვლეულია 100 პაციენტი ყბა-სახის მიდამო მწვავე ანთებითი ოდონტოგენური დაავადებით. ავადმყოფები

ტრადიციულ მედიკამენტურ მკურნალობასთან ერთად დამატებითად დებულობდნენ რეგიონულ ლიმფურ ანტიბიოტიკულ თერაპიას. ანტიბიოტიკის რეგიონული ლიმფოტროპული გზით შეყვანა ყბა-სახის და კისრის მიდამოებში ფლეგმონების მკურნალობის დროს იწვევს ჭრილობითი პროცესის მეორე ფაზის განვითარებას და უზრუნველყოფს ორგანიზმის სწრაფ დეტოქსიკაციას.

VALIDATION OF THE DIAGNOSTIC AND TREATMENT COMPLEX FOR PATIENTS WITH ORTHOGNATHIC DEFORMITIES AND PHONETIC DISORDERS

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Recently, there has been a tendency for the growth of dentognathic deformities of various origins, accompanied by phonetic abnormalities [1-4]. They can be caused by the disturbance of the dentognathic relationship, changes in the functional activity of the dentofacial muscles, the state of the articulation apparatus, ENT diseases, etc. [5-9].

These factors are in a cause-effect relation and require a simultaneous systematic multidimensional approach to the diagnosis and treatment of patients with dentognathic deformities and phonetic disorders [1-3,10-12].

To date, for this category of patients, various methods of orthodontic treatment of dentognathic deformities are used in combination with myogymnastics, teiping, as well as certain types of correctional speech therapy [1,2,13,14]. In this sense, it is of particular importance to determine the sequence, staging, volume, and duration of diagnostic and therapeutic measures to obtain the most effective end result.

The use of well-known methods of orthodontic treatment without directed speech therapy correction reduces the effectiveness of rehabilitation of patients with dentognathic deformities, which are accompanied by phonetic disorders. Attempts to normalize the dental occlusion without speech therapy and functional muscle restructuring complicate orthodontic treatment and make it impossible to achieve a stable result [6].

Given the above, there is a need for an in-depth study of the relationship between speech impairment disorders of speech function and deformities of the dentognathic apparatus, development of modern preventive, diagnostic and therapeutic measures based on a multidisciplinary approach to overcoming the problems identified.

Aim – to increase the effectiveness of orthodontic treatment of dentognathic deformities, accompanied by phonetic disorders, by developing and justifying a set of diagnostic and therapeutic measures based on a multidisciplinary approach.

Material and methods. The influence of the state of ENT organs on the formation of dentognathic deformities and phonetic disturbances is studied in 155 children who underwent rhinoscopy, pharyngoscopy, cone-beam computed tomography.

A clinical dental examination is performed in 82 patients aged 6-12 years with dentognathic deformities and phonetic disorders according to the conventional scheme with the use of objective and additional research methods. Thirty-eight patients were admitted for orthodontic treatment with distal deep, 16 – with distal, 18 – with open, 10 – with mesial bite (n = 82) with the use of removable (Schwarz', Andresen-Haupl's, Bruckl's-Reeykhenbakh, Flis P.- Filonenko V.) and non-removable (Marco Rossa) orthodontic appliances [15-17] for 10-12 months following retention period.

Efficiency of orthodontic treatment was estimated based on the results of anthropometric measurements using 3Shape Viewer software on scanned models of upper and lower jaws in 82 patients with determination of the length of the anterior segment of the tooth rows by M. Mirgazizov's method (n=328), transversal sizes of the tooth rows by Moorrees method before treatment in all 82 patients (n=164), after - in 71 (n=142), as 11 patients lost their temporary canine teeth as a result of physiological change of teeth.

A cephalometric study was conducted for 45 patients (n=90) by A. Schwarz method using the RadiocefStudio2 computer program with superimposition of cephalometric images according to the structural landmarks of the supraorbital plane CI-RO and Ce.

The volume of the upper respiratory tract (n=60) and topographic indices of the tongue (n=30) was determined using cone-beam computed tomography of the skulls of 30 children with dentognathic deformities. The obtained data were processed in the SIMPlant graphic dental program (Materialize Software, Belgium) with the construction of multiplanar, panoramic and 3D reconstructions. Cone-beam computed tomography was also used to determine the topographic parameters of the tongue in 10 children without orthodontic pathology, but with diseases of the ENT organs.

The functional state of the anterior surface of the m. masseter, front bundles of the m. temporalis, m. sternocleidomastoideus, anterior belly m. digastricus, m. orbicularis oris in 44 patients with dentognathic deformities and phonetic disorders (n = 440) was determined by the method of total (surface) electromyography using an eight-channel electromyograph "BioEMG III" company "BioResearch Inc." (USA). The state of physiological rest, volitional contraction and swallowing were subject to analysis.

Diagnosis of the phonological side of speech was carried out in 155 patients without phonetic disorders (73 people) and with deformities of the dentognathic apparatus (82 people). The indicators of sound pronunciation, the ratio of the most frequently detected distortions of sounds, the average number of violations of sound pronunciation per child in the age groups of 6-8 and 9-12 years are determined.

Individual corrective speech therapy work has been carried out to overcome the defects of the phonological side of speech, children without orthodontic pathology have been assigned 10 sessions of speech therapy three times a week, with deformities of the dentognathic apparatus and speech disorders - 2-3 courses of 10 sessions with breaks for 1-2 months.

In 12 children without orthodontic pathology, in addition to speech therapy, Dr. Hinz's MUPPY-P vestibular plates with beads were used, in 8 children – removable devices with beads, in 9 children – fixed Bluegrass appliances, in 6 children – the proposed fixed device for elimination and prevention of harmful language habits and training of muscle structures of the articulation apparatus [18,19].

Mathematical and statistical research. Statistical processing of the obtained results was carried out with the help of the mathematical program of medical and biological statistics STATISTIÑA 6.0. Statistica. The calculated parameters and correlation coefficients had equal reliability within the limits allowed for the processing of medical researches (<0, 05).

Results and their discussion. Among 82 children with deformities of the dentognathic apparatus, which are accompanied by phonetic disorders, hypertrophy of the nasopharyngeal tonsils (adenoid vegetation) of the first degree was diagnosed in 51.2% (n=42), II degree – in 30.5% (n = 25), III degree – in 18.3% (n=15). That is, in all examined patients with dentognathic de-

formities, hypertrophy of the nasopharyngeal tonsils was observed. Among 73 children without orthodontic pathology, but with speech pathology, adenoid growths of the first degree were detected in 43.8% (n = 32), II degree – in 20.5% (n = 15), and III degree – in 2.7% (n = 2). An increase in the size of nasopharyngeal tonsils in 32.9% (n = 24) without orthodontic pathology was not detected.

Adenoid growths of I degree in children with existing orthodontic pathology are observed 1.2 times more often than in children without orthodontic pathology, II degree – 1.5 times, III degree – 6.8 times. No children without an imbalance of the size of the nasopharyngeal tonsils were observed among persons with dentognathic deformities.

When conducting pharyngoscopy with determination of the size and condition of the tonsils in 82 children with dentognathic deformities, accompanied by phonetic disorder, I degree hypertrophy was found in 42.7% (n=35), II degree – in 32.9% (n=27), III degree – in 24.4% (n=20). A various degree of hypertrophy of the tonsils was recorded in all examined patients with dentognathic deformities. Among 73 children without orthodontic pathology with speech pathology, an increase in palatine tonsils of the first degree was detected in 38.4% (n=28), II degree – in 16.4% (n=12), and III degree – in 6.8% (n=5). Violation of the size of the tonsils in 38.4% of children (n=28) without orthodontic pathology was not detected.

Analysis of the state of palatine tonsils indicates that hypertrophy of the first degree in children with orthodontic pathology is 1.1 times more frequent than in children without it, II degree – 2 times, III degree – 3.6 times, respectively. The obtained data showed the presence of the relationship between dentognathic deformities and inflammatory diseases of ENT organs, which is an additional factor in changes in the volume of the upper respiratory tract, which in turn affects the location of articulation zones, speech breathing and contributes to the development of sound pronunciation disturbances.

A certain pathological “chain” of cause-effect relationships of dentognathic deformities with phonetic disorders and diseases of the ENT organs became the basis for a multidisciplinary approach to solving the problems identified. A complex of diagnostic and treatment measures for patients with dentognathic deformities accompanied by phonetic disorders, consisting of motivational, diagnostic and treatment blocks, has been developed and introduced into practice [20].

The motivational block is aimed at the perception of the positive result of orthodontic treatment and speech therapy by the child and his parents; creation of the atmosphere of emotional comfort between the patient, orthodontist, speech therapist, otolaryngologist, children’s therapist and dentist surgeon; formation of the child’s personalized treatment and correctional training.

Diagnostic activities included surveys to determine the extent of treatment interventions and to assess their effectiveness. If the parents and the child turn to an orthodontist, clinical examinations, anthropometric measurements of scanned models of the upper and lower jaws, electromyography, orthopantomography, cephalometry, and photometry have been carried out, and complaints about ENT and sound disorders have been collected, followed by a consultation with an otolaryngologist and a speech therapist; a consultation with a pediatric dentist to determine the hygienic state of the oral cavity, the intensity of dental caries, the presence of inflammatory processes of periodontal tissue and oral mucous membranes; a consultation with a pediatric surgeon dentist to identify congenital defects in the frenulum of the lips, tongue, etc. The otolaryngologist collected anamne-

sis, an examination of nasopharyngeal and palatine tonsils using rhinoscopy and pharyngoscopy. Cone-beam computed tomography with multiplanar, panoramic and 3D reconstructions was performed according to the orthodontist’s recommendation. The speech therapist determined the state of formation of the phonetic side of speech with the help of neuropsychological and speech therapy tests, reviewed the components of the articulation apparatus (tongue, lips, soft sky, bite), screening of pre- and postnatal factors of somatic health affecting the development of speech disorders.

The treatment unit included orthodontic treatment using removable and non-removable orthodontic apparatus, depending on the type of deformity, the age of the patient, the degree of formation of the dentognathic apparatus, etiology; phonetic correction with general obligatory (orofacial gymnastics, formation of speech breathing) and correctional directed (setting automation and differentiation of sounds) tasks; otolaryngological conservative and / or surgical treatment of adenoiditis and tonsillitis; control of the level of oral hygiene, therapeutic treatment of diseases of hard tooth tissues, inflammatory processes of periodontal tissues and oral mucosa; elimination of congenital defects of the frenulum of the lips, tongue, etc. This contributed to the implementation of a comprehensive multi-vector treatment of dentognathic deformities, accompanied by phonetic disorders.

After orthodontic treatment, anthropometric measurements of scanned jaw models using the M. Mirgazizov method (Fig. 1 a, b) and Moorrees method (Fig. 2 a, b) showed a change in the size of the dental row. The statistically significant reduction of the length of the frontal part of the upper tooth row was noted when treating patients with distal bite by 2.51 ± 1.39 mm, distal deep bite – by 1.06 ± 1.05 mm, and at mesial bite – the reduction of the lower one by 1.72 ± 1.79 mm and the increase of the upper tooth row by 3.43 ± 1.36 mm; enlargement in the area of canines on the upper jaw of patients with distal bite by 3.32 ± 1.03 mm and distal deep bite – by 2.59 ± 1.04 mm in comparison with the initial clinical picture.

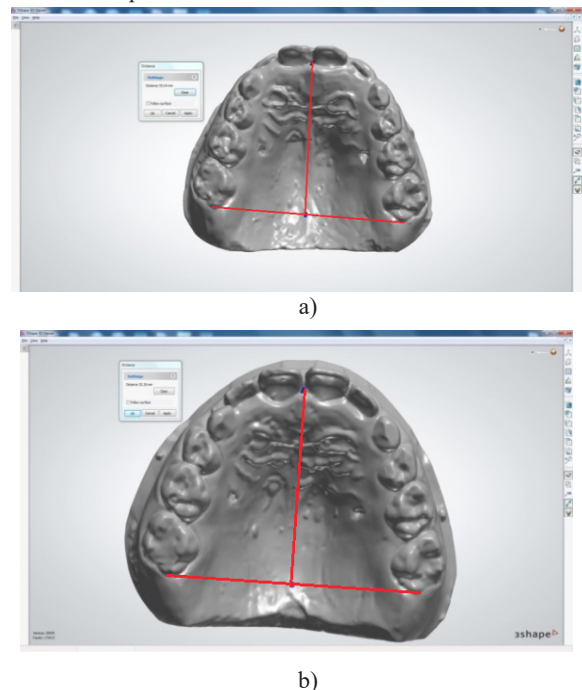


Fig. 1 Anthropometric measurements using the M. Mirgazizov method on scanned models of the upper jaw of patient L. at the beginning of treatment (a) and at the end of treatment (b)

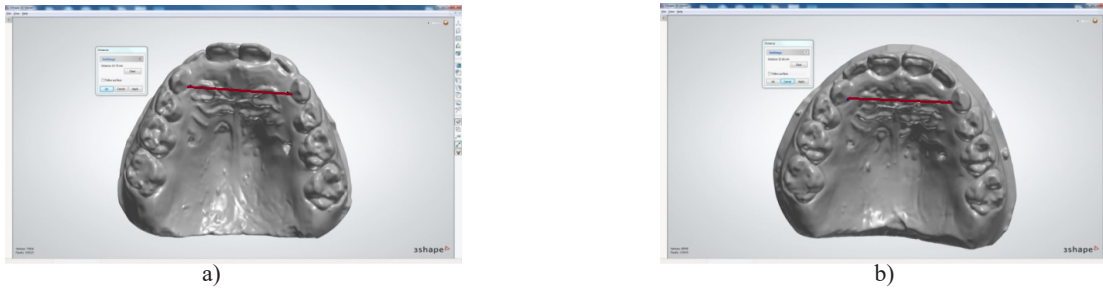


Fig. 2 Anthropometric measurements using the Moorrees method on scanned models of the upper jaw of patient L. at the beginning of treatment (a) and at the end of treatment (b)

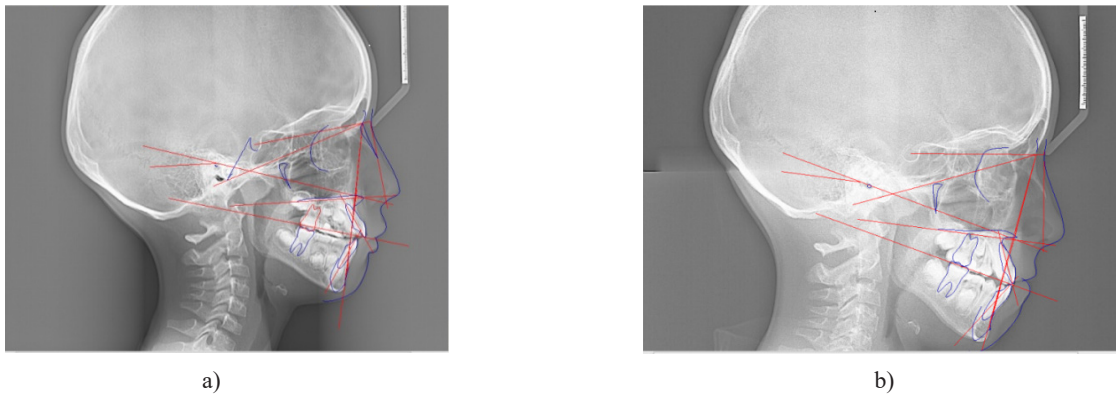


Fig. 3. Patient B. cephalogram with open bite before (a) and after (b) treatment

Analysis of cephalograms using the A. Schwarz method confirms positive changes at the end of orthodontic treatment. The most informative improvements in the placement of the apical base of the lower jaw in relation to the base of the skull in the sagittal direction in the treatment of distal and distal deep bites, the vertical position of the jaw in the treatment of open bite.

The most informative improvements in the placement of the apical base of the lower jaw relative to the base of the skull in the sagittal direction (angle SeNB), by almost 5°, were found in the treatment of distal and distal deep bites. While treating an open bite, the vertical relative position of the jaws (angle B) changed by almost 9°. The inclination of the axes of the teeth (angle 1SpP, angle 1MP) relative to the planes of the base of the jaw improved (Fig. 3 a, b, 4).

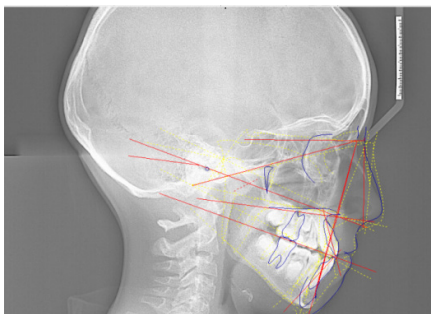


Fig. 4 Superimposition of patient B. cephalogram with open bite before and after treatment

By means of cone-beam computed tomography it is objectively proved the increase of the upper respiratory tract volume by $53.80 \pm 4.21\%$ in patients with 11.82 ± 2.06 ml up to 18.01 ± 3.84 ml after enlargement of the upper jaw at the dentoalveolar level, which leads to a change in the position of the tongue with its dislocation to the hard palate, thus improving the results of orthodontic treatment and creating optimal conditions for effective correction of sound pronunciation (Fig. 5 a, b, c, 6 a, b, 7 a, b, 8 a, b).

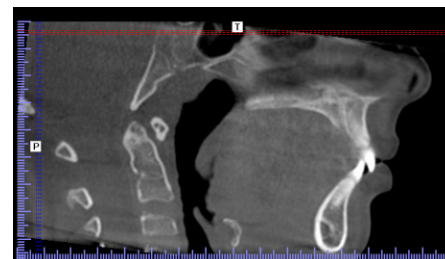


Fig. 5 Coronary section of a cone-beam computed tomogram of patient C. airways, before (a) and after treatment (b), overlay of the airway profile before and after treatment (c)

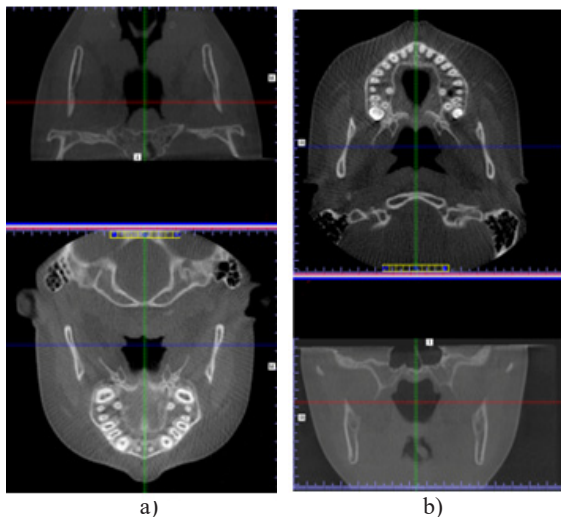


Fig. 6 Axial and frontal sections of cone-beam computed tomography of the respiratory tract of patient C. before (a) and after treatment (b)

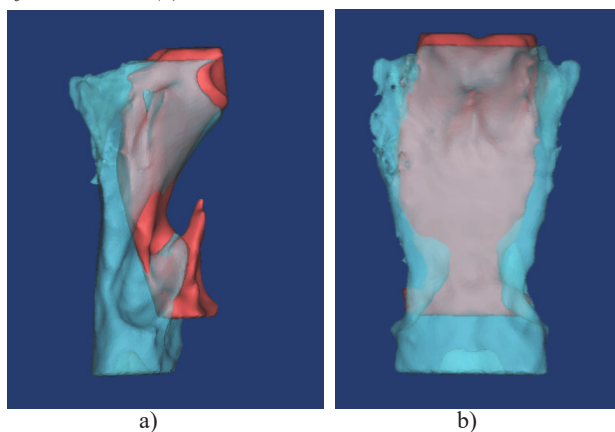


Fig. 7 3D reconstruction of the upper respiratory tract and superposition of patient C. volumes (prior to treatment – volume reconstruction in red, after completion of treatment – blue, side view (a), front view (b))

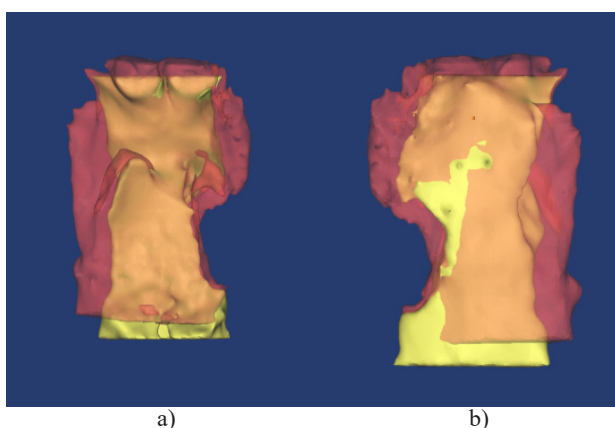


Fig. 8 3D reconstruction of the upper respiratory tract and superposition of patient P. volumes (before treatment, volume reconstruction in red, after treatment, yellow, side view (a), front view (b))

The determination of the volume of the respiratory tract is a clear criterion for evaluating the effectiveness of orthodontic treatment and creating conditions for speech therapy correction.

Changes in the indicators of the biopotentials of the muscular

structures of the articulatory apparatus depending on the forms of dentognathic deformities, accompanied by phonetic disturbances, confirm their correlation. The given results of electromyographic analysis of the condition of the surface parts of the m. masseter, front bundles of the m. temporalis, m. sternocleidomastoideus, anterior belly of the m. digastricus, and m. orbicularis oris before orthodontic treatment indicate the group of muscles with the greatest dysfunction in accordance with the established orthodontic pathology and direct the work of the orthodontist and speech therapist to restore the neuromuscular balance of this particular group.

Patients with mesial bite before orthodontic treatment at rest registered an increase in the bioelectric activity of the front bundles m. temporalis (7.81 ± 2.07 mV) and the m. masseter (2.29 ± 1.03 mV) and a significant decrease in the contractile activity of the latter at will compression (19.94 ± 7.37 mV); with open – the amplitude of biopotentials of the upper part of m. orbicularis oris was reduced at rest (3.21 ± 1.07 mV) and at will compression (9.25 ± 2.38 mV), the increase of bioelectric activity of the front bundles of the m. temporalis, at rest (4.62 ± 1.13 mV); in distal deep and distal, the amplitude of biopotentials of the upper part of m. orbicularis oris in a resting state is the highest (5.24 ± 1.17 mV and 5.04 ± 2.01 mV, respectively).

After the orthodontic treatment, along with the speech therapy correction, changes in the electromyogram parameters were established, which showed an improvement in the functional state of the muscles and the effectiveness of the treatment by an average of 2.5 times. In patients with a mesial bite in a resting state, a decrease in the bioelectric activity of the front bundles of the m. temporalis (2.11 ± 0.97 mV) and of the m. masseter (1.32 ± 0.78 mV), an increase in their contractile activity during volitional compression was recorded (44.48 ± 6.33 mV); open – an increase in the amplitude of the biopotentials of the upper part of m. orbicularis oris with volitional compression (12.84 ± 3.51 mV), a decrease in the bioelectric activity of the front bundles of the m. temporalis at rest (0.87 ± 0.22 mV) in the distal deep and distal – a decrease in the amplitude of the biopotentials of the upper part of m. orbicularis oris at rest (3.22 ± 1.37 mV and 2.76 ± 1.02 mV, respectively).

The topographic indices of the tongue as a powerful muscle factor of influence on the development of the dentognathic apparatus and sound pronunciation in children are determined. Analyzing cone-beam computed tomograms it was found that an increase in its size leads to changes in the anatomical and topographic relationship with surrounding tissues. The ratio of the thickness of the front, middle and back third of the tongue in children aged 8-12 years is 1:1.7:1.8, respectively. A change in any parameter can directly or indirectly affect an increase in its asymmetry, which is one of the reasons for the formation of dentognathic deformities. The asymmetry of the left and right halves of the tongue was within 5% in children without orthodontic pathology and 15% in children with intermaxillary interrelation disorders.

Diagnostic screening of sound pronunciation disorders was carried out and their dependence on dentognathic deformitis was established. The average number of violations of the pronunciation of whistling sounds per child is greatest with a mesial bite at the age of 6-8 years – 2.4 and with an open bite at the age of 9-12 years – 2.0 and sonant in distal deep at the age of 6-8 years – 1.7, at the age of 9-12 years – 1.1. The ratio of sound pronunciation disorder of sonant to whistling at the mesial bite at the age of 6-8 years is 6.0, at the open bite – 4.7, at the age of 9-12 years, respectively, 4.5 and 3.0. The lowest average number of

disorders of hissing at the age of 6-8 years – 0.4, at the age of 9-12 years – 0.6 per child at all bite pathologies is determined. A step-by-step work was carried out to overcome phonetic speech disorders, which contributed to the normalization of the sound pronunciation of whistling, sonant and hissing sounds in various types of dentognathic deformities.

The expediency and effectiveness of the proposed fixed device (Fig. 9), which helps to train the muscle structures of the articulation apparatus in combination with speech therapy correction, to improve the kinetic and kinesthetic praxis in the temporary and first period of the mixed occlusion, has been proved [19]. This is due to a decrease in the size of the device structure, an increase in articulation zones, and facilitation of hygienic care.



Fig. 9 Device for elimination and prevention of harmful oral habits

The device consists of a bracket on which a functionally active bead-shaped element is attached in the middle part of the device; the bracket is soldered to two thin-walled cast perforated crowns intended for fixation on temporary canines. The technical result is as follows: the fixation on the temporary canines makes the device compact by reducing the size of the construction, which in turn facilitates hygienic care and improves oral hygiene; the compactness increases the articulation zones of the tongue, which in turn has a positive effect on the quality of speech therapy treatment. The use of a functionally active bead-shaped element makes it possible to control the usual palatal position of the tongue, to activate the work of the tongue root, as the child unwittingly rolls it in the palate, stimulating the tongue muscles.

Evaluation of the effectiveness of the diagnostic and treatment complex of measures proposed for the patient with dentognathic deformities accompanied by phonetic disorders, based on the multidisciplinary approach, showed the need to determine the condition of the nasopharyngeal and palatine tonsils and proved the effectiveness of orthodontic treatment along with speech therapy correction accompanied by a pediatric therapist and surgeon. There was an improvement in electromyography, anthropometric measurements of scanned jaw models, cephalometry in 86.6% of patients; analysis of cone-beam computed tomography data confirmed a significant increase in the volume of the upper respiratory tract by $53.8 \pm 4.2\%$ in patients after the orthodontic treatment, which in turn contributed to corrective speech therapy.

Conclusions. The etiopathogenetic relationship of otolaryngological diseases of an inflammatory nature with dentognathic deformities was determined. In children with orthodontic pathology, hypertrophy of the tonsils of the third degree was more often manifested (6.8 times nasopharyngeal and 3.6 times palatine) than in children without dentognathic deformities. The obtained data showed the presence of a relationship between dentognathic deformities and inflammatory diseases of the ENT organs, which are factors leading to a decrease in the volume of

the upper respiratory tract to 11.82 ± 2.06 ml, changes in articulation zones, speech breathing and affect the pronunciation of the sound. They are the basis for the prescription of otolaryngological conservative and/or surgical treatment of hypertrophied nasopharyngeal and palatine tonsils.

The qualitative and quantitative dependence of sound deterioration on the type of dentognathic deformities is established. After individually directed speech therapy correction with the use of orofacial gymnastics the indicators of sound pronunciation of whistling and sonorous sounds approached the norm. In combination with speech therapy correction for improvement of kinetic and kinesthetic praxis the fixed device which promotes the training of muscular structures of the articulation apparatus is offered. The expediency of its use in the temporary and first period of the mixed occlusion is confirmed.

The proposed complex of diagnostic and treatment measures made it possible to increase the efficiency of orthodontic treatment of children with dentognathic deformities with disturbances of sound pronunciation depending on the type of bite by means of a multidisciplinary approach involving an otolaryngologist, speech therapist, children's therapist and surgeon, which was confirmed in 86.6% of patients by the improvement of electromyography, anthropometric measurements of scanned models of jaws, cephalometry; the analysis of cone-beam computed tomography data showed a significant increase in the upper respiratory tract volume by $53.8 \pm 4.2\%$.

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SUMMARY

VALIDATION OF THE DIAGNOSTIC AND TREATMENT COMPLEX FOR PATIENTS WITH ORTHOGNATHIC DEFORMITIES AND PHONETIC DISORDERS

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Recently, there has been a tendency for the growth of dentognathic deformities of various origins, accompanied by phonetic abnormalities.

Aim – to increase the effectiveness of orthodontic treatment of dentognathic deformities, accompanied by phonetic disorders, by developing and justifying a set of diagnostic and therapeutic measures based on a multidisciplinary approach.

The influence of the state of ENT organs on the formation of dentognathic deformities and phonetic disturbances is studied in 155 children. A clinical dental examination and orthodontic treatment is performed in 82 patients aged 6-12 years. Individual corrective speech therapy work has been carried out to overcome the defects of the phonological side of speech.

A certain pathological “chain” of cause-effect relationships of

dentognathic deformities with phonetic disorders and diseases of the ENT organs became the basis for a multidisciplinary approach to solving the problems identified. The qualitative and quantitative dependence of sound deterioration on the type of orthognathic deformities is established. A complex of diagnostic and therapeutic measures for patients with dental deformities accompanied by phonetic disorders, consisting of motivational, diagnostic and therapeutic blocks, has been developed and introduced into practice.

The proposed complex of diagnostic and treatment measures made it possible to increase the efficiency of orthodontic treatment of children with dentognathic deformities with disturbances of sound pronunciation depending on the type of bite by means of a multidisciplinary approach involving an otolaryngologist, speech therapist, children’s therapist and surgeon, which was confirmed in 86.6% of patients by the improvement of electromyography, anthropometric measurements of scanned models of jaws, cephalometry; the analysis of cone-beam computed tomography data showed a significant increase in the upper respiratory tract volume by 53.8±4.2%.

Keywords: children, dentognathic deformities, phonetic disorders, diagnosis, treatment, multidisciplinary approach.

РЕЗЮМЕ

ОБОСНОВАНИЕ КОМПЛЕКСА ДИАГНОСТИЧЕСКИХ И ЛЕЧЕБНЫХ МЕРОПРИЯТИЙ ДЛЯ ПАЦИЕНТОВ С ЗУБОЧЕЛЮСТНЫМИ ДЕФОРМАЦИЯМИ И ФОНЕТИЧЕСКИМИ НАРУШЕНИЯМИ

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В последнее время наблюдается тенденция роста зубочелюстных деформаций различного происхождения, сопровождающихся фонетическими нарушениями.

Цель исследования - повышение эффективности ортодонтического лечения зубочелюстных деформаций, сопровождающихся фонетическими нарушениями, путем разработки и обоснования комплекса диагностических и лечебных мероприятий на основании мультидисциплинарного подхода.

Влияние состояния ЛОР-органов на формирование зубочелюстных деформаций и фонетических нарушений изучено у 155 детей. Клиническое стоматологическое обследование и ортодонтическое лечение проведено 82 пациентам в возрасте 6-12 лет. Выполнена индивидуальная корректирующая логопедическая работа по преодолению дефектов фонологической части речи.

Предложенный комплекс диагностических и лечебных мероприятий позволил повысить эффективность ортодонтического лечения детей с деформациями зубочелюстного аппарата и нарушениями речи, в зависимости от типа прикуса с помощью междисциплинарного подхода с участием отоларинголога, логопеда, детского терапевта и хирурга, что подтверждено улучшением показателей электромиографии, антропометрических измерений сканированных моделей челюстей, цефалометрии у 86,6% пациентов; анализ данных конусно-лучевой компьютерной томографии показал увеличение объема верхних дыхательных путей на 53,8±4,2%.

რეზიუმე

პაციენტების სადიაგნოსტიკო და სამკურნალო ღონისძიებების კომპლექსის დასაბუთება პაციენტებისათვის ყბა-კბილთა დეფორმაციებით და ფონეტიკური დარღვევებით

პ.ფლისი, ლ.იაკოვენკო, ვ.ფილონენკო, ა.მელნიკი

ა.ბოგომოლცის სახელობის ეროვნული სამედიცინო უნივერსიტეტი, კიევი, უკრაინა

კვლევის მიზანს წარმოადგენდა ყბა-კბილთა დეფორმაციების და თანხვედრილი ფონეტიკური დარღვევების ოთოლოგიური მკურნალობის ეფექტურობის გაუმჯობესება სადიაგნოსტიკო და სამკურნალო ღონისძიებების კომპლექსის მულტიდისციპლინური მიდგომით შემუშავებისა და დასაბუთების საშუალებით.

ყელ-ყურ-ცხვირის ორგანოთა მდგომარეობის გაეყენა ყბა-კბილთა დეფორმაციების და თანხვედრილი

ფონეტიკური დარღვევების განვითარებაზე შესწავლილია 155 ბავშვზე. კლინიკური სტომატოლოგიური გამოკვლევა და ოთოლოგიური მკურნალობა ჩატარდა 6-12 წლის ასაკის 82 პაციენტს. მეტყველების ფონოლოგიური ნაწილის დეფექტების აღმოფხვრისათვის ჩატარებულია ინდივიდუალური მაკორეგირებელი ლოგოპედიური სამუშაო.

სადიაგნოსტიკო და სამკურნალო ღონისძიებების შეთავაზებული კომპლექსი იძლევა ყბა-კბილთა დეფორმაციების და თანხვედრილი მეტყველების დარღვევების მქონე ბავშვების ოთოლოგიური მკურნალობის ეფექტურობის გაუმჯობესების საშუალებას თანკბილის ტიპის გათვალისწინებით და ინტერდისციპლინური გუნდის მონაწილეობით – ოტორინოლარინგოლოგი, ლოგოპედი, პედიატრი და ქირურგი, რაც დასტურდება პაციენტების 86,6%-ში ელექტრომიოგრაფიის, ყბების მოდელის ანთროპომეტრიული გაზომვების სკანირების და ცეფალომეტრიის მანევრების გაუმჯობესებით; კონუსურ-სხივური კომპიუტერული ტომოგრაფიის მონაცემების ანალიზმა აჩვენა ზედა სასუნთქი გზების მოცულობის გაზრდა 53,8 4,2%-ით.

EVALUATION OF GENE POLYMORPHISM OF IL-1 β AND IL-10 IN CHILDREN WITH NEPHROTIC SYNDROME

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Estimating the prevalence of chronic kidney disease (CKD) in children and investigating markers of the prognosis and progression are the priority for public health. Approximately 9 in every 1 million children in the developed world require renal replacement therapy treating end-stage renal disease of CKD. The prevalence of CKD and end-stage renal disease is growing worldwide [5,13]. Taking into account the considerable prevalence and progression of CKD in children, findings effective methods of the prevention of this disease is important goal of all pediatric nephrologists. The issue of early onset of prophylaxis, the individual factors of progression of CKD not completely understood [15].

Traditional risk factors of the progression of CKD are arterial hypertension, persistent proteinuria, anemia, congenital anomalies, progressive course of the disease and resistance to pathogenetic treatment, hereditary history, and acute renal failure [4]. Nephrologists have good results in decreasing of both progression of disease and number of patients with end-stage renal disease by avoiding or correction of risk factors of CKD, and widely using renoprotective therapy. However, despite the results, the improving of quality of life and reducing the number of patients with progressive CKD is still unresolved. Genetic factors may also influence the incidence and/or the progression of CKD and its complications. Studies of genetic factors are now interesting in this population. The goal of identifying genetic factors that contribute to the outcome of CKD is to gain further understanding of the disease pathogenesis and underlying

causes and, possibly, to use this knowledge to predict disease or its complications. Furthermore, by identifying patients' genetic backgrounds, it is possible that a more individualized therapy could be performed [1,3].

Progression of CKD in children with chronic glomerulonephritis also connected with immune inflammation which is known to be a marker of unfavorable prognosis. Chronic glomerulonephritis is considered as immunocomplex disease in which monocytes are activated and secreted a wide variety of biologically active compounds into the blood.

The immune inflammation is a cascade of biochemical and immunological reactions regulated by a large number of mediators, among which a special place is belonged to cytokines – low-molecular weight proteins. Each cytokine has cross-linked, synergistic or inhibitor activity in relation to other cytokines.

It is known that in the development of glomerular injury and nephrosclerosis great role belongs to pro-inflammatory cytokine IL-1 β [10]. It has been shown that IL-1 β is a key cytokine that induces the development of a cascade of other proinflammatory cytokines. This leads to glomerular and tubulointerstitial damage and stimulates the fibrogenesis of nephrons. IL-1 β is considered to be one of the factors of progression of chronic glomerulonephritis. Balance between the production, expression and inhibition of the synthesis of proteins of the IL-1 family play the main role in the development of any inflammatory process. It was known that a higher level of IL-1 was determined in part of the patients, even before identifying the association of increased

IL-1 production with certain alleles. It has also been shown that the duration and intensity of the inflammatory process in different individuals may be different [4].

The predisposition to multifactorial diseases, especially their course, the effectiveness and safety of their treatment are largely determined by a specific set of polymorphic variants of genes.

The aim of our work was to determine the gene polymorphism of cytokines IL-1 β (-511) and IL-10 (-1082) in children with nephrotic syndrome.

Material and methods. 20 patients with nephrotic syndrome were recruited into the study from 2017 to 2018 years in single center (Vinnytsya regional clinical children's hospital), in Ukraine. Mean age of the patients was 11,73 \pm 3,63 years. Boys and girls were met with the same frequency among them. All patients were diagnosed with nephrotic syndrome and received subsequent medical care at Vinnytsya regional clinical children's hospital.

All the children were carefully clinically and laboratory examined. Blood and urine samples were collected after 8 h of overnight fast. Our study included children with levels of glomerular filtration rate >90 ml/min. Estimated glomerular filtration rate (eGFR) was calculated using the Schwarz formula.

Genetic polymorphism of IL-1 β (-511) and IL-10 (-1082) and serum IL1 β were evaluated. The variants of IL-1 β and IL-10 genes were determined from whole blood samples. Polymorphic analysis for IL-1 β and IL-10 was performed by polymerase chain reaction (PCR) and subsequent restriction fragment length polymorphism (RFLP) methods. The level of IL-1 β was determined by the ELISA method using standard reagent kits.

Statistical analysis was performed using the SPSS software. Difference between different groups of patients was analyzed by Mann-Whitney U-test. P values < 0.05 were considered to be statistically significant.

The study was performed in accordance with the Declaration of Helsinki and was approved by local ethics committees at the individual study center. All patients and their parents gave written informed consent before undergoing any study related procedures.

Results and their discussion. Of the 20 patients, 4 were diagnosed as nonrelapsers or infrequent relapsers of nephrotic syndrome, 12 patients as frequent relapsers, and 4 with steroid resistant nephrotic syndrome according to the diagnostic criteria of the KDIGO recommendations. All the children had I stage of CKD without renal insufficiency. There were 12 children in remission of disease during examination, 8 children had active stage of nephrotic syndrome.

We determined the variants of IL-1 β (-511) and IL-10 (-1082) genes in children with nephrotic syndrome (Table 1).

We found the prevalence of the genotype CT (16 patients – 80%) of allelic polymorphism of IL-1 β (-511) gene. Among the patients with nephrotic syndrome 4 had the genotype CC of the gene IL-1 β (-511) (20 %).

Preliminary evidence primarily from adult CKD studies indicates that gene polymorphisms of IL-1 β CT (-511) was associated with the progressive course of CKD. Presence of the C/T allelic polymorphism of the gene IL-1 β (-511) in children with glomerulonephritis in the human genome can be one of the factors of the progression of CKD.

We analyzed allelic polymorphism of IL-10 as one of the important anti-inflammatory interleukins. IL-10 attenuates the inflammatory response. Decreased production of IL-10 is associated with increased CRP. Checking the polymorphism of SNP -1082 of IL-10, we determined that in 50% of children with nephrotic syndrome there was G/A genotype, in 40% - G/G genotype, and genotype A/A was only in 10% of patients. The low producer genotype A/A of the gene -1082 G>A SNP is associated with increased cardio-vascular mortality in end-stage renal disease patients.

Analyzing the contents of IL -1 β in serum of children with chronic glomerulonephritis, nephrotic syndrome, we found that IL -1 β was significantly increased in children with steroid-resistant nephrotic syndrome and with progression of glomerulonephritis compared with remission and with healthy children (p<0.05). The presence of C/T genotype is associated with twice increased production of interleukin-1 β in serum, compared with children with genotype C/C (p<0.05) (Table 2).

A strong direct relationship between the level of IL-1 β in serum and C/T allelic polymorphism of the gene IL-1 β (-511) was found (r=+0,56) (p<0.05). This indicate an increased level of secretion of this interleukin in the presence of C/T genotype of IL -1 β .

We analyzed connection between allelic polymorphism of gene of IL-1 β and the course of the disease in our patients. Patients were divided into 3 groups: nonrelapsers or infrequent relapsers of nephrotic syndrome, frequent relapsers, and with steroid resistant nephrotic syndrome in remission and in active stage. 4 children with steroid resistant nephrotic syndrome had C/T allelic polymorphism of the gene IL-1 β (-511). C/C allelic polymorphism of the gene IL-1 β (-511) mostly was found in children in remission of glomerulonephritis. Our data suggest that genetic association studies have the potential to provide new insights into the factors responsible for CKD progression in case of nephrotic syndrome.

Recently, several studies have reported that polymorphisms of cytokine genes were associated with the development and severity of inflammatory diseases. Many researchers have evaluated the association with genetic polymorphisms in the IL-1b, IL-1ra, and TNF-a genes in patients with various inflammatory diseases, including IgA nephropathy, ankylosing spondylitis, and multiple sclerosis [7,9,11,13]

Interleukin genes have an extremely high degree of polymorphism, and the number of sites of this polymorphism in one gene can reach several dozen and they can be located both in encoding exons and introns, and, most importantly, in the promoter

Table 1. Allele frequencies of IL-1b and IL-10

Group of investigation	IL-1 β (-511)		IL-10 (-1082)		
	CT	CC	GA	GG	AA
Children with nephrotic syndrome	16(80%)	4(20%)	10(50%)	8(40%)	2(10%)

Table 2 Levels of IL-1 β according to gene polymorphism

	Genotype CC	Genotyp CT	Healthy children
IL-1 β (pg/ml)	4,65 \pm 0,27	10,66 \pm 0,96*	3,16 \pm 0,25

regulatory regions of the gene structure. These DNA regions contain zones of binding of regulatory factors that determine not the reading structure, but the intensity of the end-product protein production, that is, the molecules of interleukins itself. The presence of allelic polymorphism in the promoter regions of the genes of interleukins provides a variety of individuals according to the cytokine production.

It is known that each gene is located in one of 23 pairs of chromosomes. The two alleles may be the same or differ from each other. Polymorphism represents variants of alleles that are relatively common in the population and are generally associated with a deviation in the expression or function of enzymes. Genetic polymorphism is a nucleotide variation in a particular genomic sequence, including insertions, deletions, single nucleotide polymorphism (SNP), which account for about 90% of all variations in the genome.

The genes encoding IL-1 β are localized on the chromosome 2q 13-21. Among the allelic polymorphisms of IL-1 β , the most studied are changes in the positions -511, -31, +3953, representing the replacement of one nucleotide. Analysis of transcriptional activity showed that at position -511 cytosine is replaced by thymine (C \rightarrow T), and at position-31 thymine is replaced by cytosine (T \rightarrow C). There are studies about the relation of polymorphism in the position -511 C \rightarrow T with a progressive and more severe course of nephropathy. It is proved that polymorphic variants of the IL-1 β gene are highly productive. Persons who are homozygous for the high-producing IL-1 β allele are produced 4 or 2 times more of this cytokine, respectively, in comparing the homozygous individuals with non-mutant allele of this gene. It has also been proven that the IL-1 β gene polymorphism has a close relationship with such effects as hypertension, atherosclerosis, cardiovascular complications, progression of nephropathy [2,6].

Violation of the synthesis of IL-10 may play an important role in the pathogenesis of glomerulonephritis. IL-10, as one of the anti-inflammatory cytokines, reduces inflammatory reaction. Pathological decline in the production of this interleukin leads to an increase in C-reactive protein. The genes encoding IL-10 are localized on the chromosome 1q 31-32. Among the polymorphisms of the gene most studied are -592 C \rightarrow A, -819 C \rightarrow T and -1082 G \rightarrow A. In different studies, changes -1082 G \rightarrow A are associated with mortality from cardiovascular complications in patients with an end-stage renal disease. In a number of studies, it has been established that the genotype A/A in the allelic polymorphism -1082 G \rightarrow A leads to decreased production of IL-10 and increased levels of cardiovascular morbidity.

Recent study with investigating of the association between single nucleotide polymorphisms of the IL-1 gene cluster and childhood IgA nephropathy suggested that the IL1 β and IL1RN genes are associated with increased susceptibility to IgAN in children. They also suggested that the development of proteinuria in IgAN is related to IL1A and that podocyte foot process effacement is associated with IL1 β [8,13].

In another study, they investigated whether genetic polymorphisms of TNF- α , IL-1 β , IL-6, and IL-10 genes leading to a more intense inflammatory response might predispose very low birth weight infants to the development of acute renal failure in severe infection. The single presence of TNF- α , IL-1 β , IL-6, and IL-10 variants does not influence the development of acute renal failure, but the constellation of TNF- α and IL-6 genetic variants is associated with acute renal failure [14].

Unfortunately, studies of IL-1b, IL-1ra, and TNF-a gene polymorphism are limited in children with nephrotic syndrome [12].

Conclusions. Our data suggest the prevalence of the genotype C/T of allelic polymorphism of IL-1 β (-511) gene in children with nephrotic syndrome. In our study, we demonstrated that pro-inflammatory cytokine IL-1 β is independently associated with C/T allelic polymorphism of the gene IL-1 β (-511) in children with steroid-resistant nephrotic syndrome and with possible progression of glomerulonephritis. Polymorphism of SNP -1082 of IL-10 consists of 50% of children with G/A genotype, 40% - G/G genotype, and 10% genotype A/A.

Genetic testing has increasingly become a valuable tool in the identification of genetic variations associated with nephrotic syndrome and may decrease the need for biopsies in the future.

Genetic screening of paediatric CKD patients may enhance the impact of preventive measures that could have a positive effect on outcome. Further studies are needed to confirm our findings and to elucidate the role of the IL-1 β gene polymorphism in the progression of CKD in children with nephrotic syndrome.

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SUMMARY

EVALUATION OF GENE POLYMORPHISM OF IL-1 β AND IL-10 IN CHILDREN WITH NEPHROTIC SYNDROME

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The aim of our work was to determine the gene polymorphism of cytokines IL-1 β (-511) and IL-10 (-1082) in children with nephrotic syndrome.

20 patients with nephrotic syndrome were recruited into the study from 2017 to 2018 years in single center. Our study included children with levels of glomerular filtration rate >90 ml/min. Genetic polymorphism of IL-1 β (-511) and IL-10 (-1082) and serum IL1 β were evaluated.

Analyzing the contents of IL-1 β in serum of children with nephrotic syndrome, we found that IL-1 β was significantly increased in children with steroid-resistant nephrotic syndrome and with progression of glomerulonephritis compared with remission and with healthy children ($p < 0.05$). The presence of C/T genotype is associated with increased production of interleukin-1 β in serum, compared with children with genotype C/C ($p < 0.05$). Checking the polymorphism of SNP -1082 of IL-10 we determined that in 50% of children with nephrotic syndrome there was G/A genotype, in 40% - G/G genotype, and genotype A/A was only in 10% of patients. A strong direct relationship between the level of IL-1 β in serum and C/T allelic polymorphism of the gene IL-1 β (-511) was found ($r = +0.56$) ($p < 0.05$).

Gene polymorphism of IL-1 β (-511) can be used as a marker of progression of glomerulonephritis, nephrotic syndrome but more studies are needed.

Keywords: children, nephrotic syndrome, cytokines IL-1 β , IL-10, gene polymorphism.

РЕЗЮМЕ

ПОЛИМОРФИЗМ ГЕНОВ ИЛ-1 β И ИЛ-10 У ДЕТЕЙ С НЕФРОТИЧЕСКИМ СИНДРОМОМ

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Целью исследования явилось определение полиморфизма генов цитокинов ИЛ-1 β (-511) и ИЛ-10 (-1082) у детей с нефротическим синдромом.

20 пациентов с нефротическим синдромом включены в исследование с 2017 по 2018 гг. В исследование были вклю-

чены детей с уровнем скорости клубочковой фильтрации >90 мл/мин. Оценивали генетический полиморфизм ИЛ-1 β (-511), ИЛ-10 (-1082) и уровень сывороточного ИЛ1 β .

Анализ содержания ИЛ-1 β в сыворотке у детей с нефротическим синдромом выявил, что ИЛ-1 β значительно повышен у детей с стероид-резистентным нефротическим синдромом и детей с прогрессирующим гломерулонефритом в сравнении с ремиссией и здоровыми детьми ($p < 0,05$). Наличие C/T генотипа связано с повышенной продукцией ИЛ-1 β в сыворотке в сравнении с детьми с генотипом C/C ($p < 0,05$). Изучая полиморфизм SNP-1082 ИЛ-10, определено, что у 50% детей с нефротическим синдромом имеется G/A генотип, у 40% - G/G генотип, а генотип A/A - у 10% пациентов. Обнаружена сильная прямая связь между уровнем ИЛ-1 β в сыворотке и C/T аллельным полиморфизмом гена ИЛ-1 β (-511) ($r = +0,56$) ($p < 0,05$).

Результаты проведенного исследования позволяют заключить, что генный полиморфизм ИЛ-1 β (-511) можно использовать в качестве маркера прогрессирования гломерулонефрита и нефротического синдрома.

რეზიუმე

ინტერლეიკინ-1 β -ს და ინტერლეიკინ-10-ის გენების პოლიმორფიზმი ბავშვებში ნეფროზული სინდრომით

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ვინიცას ნ.პიროგოვის სახელობის ეროვნული სამედიცინო უნივერსიტეტი, უკრაინა

კვლევის მიზანს წარმოადგენდა ინტერლეიკინი-1 β -ს (-511) და ინტერლეიკინი -10-ის (-1082) გენების პოლიმორფიზმის განსაზღვრა ბავშვებში ნეფროზული სინდრომით.

კვლევაში 2017-2019 წწ. ჩართული იყო 20 პაციენტი ნეფროზული სინდრომით, გორგლოვანი ფილტრაციის სიჩქარით >90 მლ/წთ. ფასდებოდა ინტერლეიკინი-1 β -ს (-511) და ინტერლეიკინი-10-ის (-1082) გენების პოლიმორფიზმი და შრატისმიერი ინტერლეიკინი-1 β .

ინტერლეიკინი-1 β -ს შემცველობის ანალიზის საფუძველზე ნეფროზული სინდრომის მქონე ბავშვებში გამოავლდა ინტერლეიკინი-1 β მნიშვნელოვანი მატება ბავშვებში სტეროიდ-რეზისტენტული ნეფროზული სინდრომით და ბავშვებში გლომერულონეფრიტის პროგრესირებით რემისიაში მყოფ და ჯანმრთელ ბავშვებთან შედარებით ($p < 0,05$). C/T გენოტიპის არსებობა დაკავშირებულია ინტერლეიკინი-1 β -ს მონატებულ პროდუქციასთან C/C გენოტიპის მქონე ბავშვებთან შედარებით ($p < 0,05$). ინტერლეიკინი -10-ის SNP-1082 პოლიმორფიზმის კვლევით დადგენილია, რომ ნეფროზული სინდრომით ბავშვების 50%-ს აქვს G/A გენოტიპი, 40%-ს - G/G გენოტიპი, 10%-ს კი - A/A გენოტიპი. დადგენილია ძლიერი პირდაპირი კავშირი სისხლის შრატში ინტერლეიკინი-1 β -ს და ინტერლეიკინი-1 β -ს (-511) გენის C/T ალელურ პოლიმორფიზმს შორის ($r = +0,56$) ($p < 0,05$).

ჩატარებული კვლევის შედეგები საფუძველს იძლევა დასკვნისათვის, რომ ინტერლეიკინი-1 β -ს (-511)-ის გენური პოლიმორფიზმი შესაძლოა გამოყენებული იყოს გლომერულონეფრიტის და ნეფროზული სინდრომის პროგრესირების მარკერად.

MICROBIOLOGICAL AND IMMUNOLOGICAL ASSESSMENT OF A COMPLEX OF THERAPEUTIC-PREVENTIVE MEASURES FOR CHRONIC CATARRHAL GINGIVITIS IN CHILDREN WITH DIABETES MELLITUS

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Dysbacteriosis of the oral cavity is known to complicate the course of chronic catarrhal gingivitis (CCG) resulting from functional disorders of the macroorganism ecosystem, decreased amount of probiotic and increased amount of opportunistic and pathogenic microflora [1-5]. And natural immune response is activated first in this case. It differentiates pathogens by means of special receptors of a wider specificity. These receptors are able to find and bind structural components of family groups of the major types of bacteria, viruses, and fungi. First of all, they include toll-like receptors of 2 and 4 types (TLR -2, -4) [6-9]. The main function of TLR is signal transmission of specific congenitally coded receptors into soluble mediators bounded with T and B-cells through the specific cytokine-chemokine receptors. Due to TLR initiation of the transcription of inflammatory cytokines, neutrophil chemoattractant, matrix metalloproteinases occurs, which destroy the periodontal tissue [8,10,11].

Scientific literature describes an important value of natural immunity in the development of [11-14]. Foreign researchers determined relations of these pathogenic chains with the development of periodontal tissue diseases [15-18]. However, these studies among children are not numerous [19,20].

A complex of treatment of CCG among children against the ground of insulin therapy according to basic-bolus scheme is suggested to be complemented with topical administration of an antiseptic solution possessing a pronounced bactericidal action concerning gram-positive, gram-negative and anaerobic microflora, as well as fungicidal effect; probiotic of immunotropic action on the basis of *L. reuteri*; an oral immune modulator of a plant origin which action is directed to stimulation of nonspecific reaction of the immune system at the expense of increased phagocytosis of macrophages and granulocytes, and intracellular destruction of bacteria in the process of phagocytosis.

The Aim of the study is to enhance the efficacy of treatment of chronic catarrhal gingivitis in children suffering from type 1 diabetes mellitus by means of improving the methods of pharmacological correction on the basis of investigation of the indices of local natural immune response and microbiological properties of the disease.

Material and methods. With the purpose to assess laboratory an improved method of treatment and prevention of CCG in children with DM we have conducted examination and treatment of 54 children aged 12 years, suffering from CCG with comorbid type 1 DM including 32 boys and 22 girls. They were divided into 2 subgroups: the main (30 individuals) and the group of comparison (24 individuals).

Microbiological inoculation of scrape taken from the oral mucous membranes was made in order to determine microflora by means of traditional bacteriological examination. The expression level of mRNA natural immunity indices in the buccal epithelium was detected by means of polymerase chains reaction with reverse transcription in the regimen of real time.

The degree of probability of the obtained results in case of normal distribution of both samples was statistically processed

according to Student-Fisher's criterion, in other cases – U-Wilcoxon for independent sampling and T-Wilcoxon for dependent sampling.

The treatment of chronic catarrhal gingivitis with comorbid DM against insulin therapy according to basic-bolus scheme included teaching hygienic individual care of the oral cavity, professional hygiene and oral cavity sanitation.

The children from the main group were suggested to rinse the oral cavity with the antiseptic solution “Decasan” 0,02% topically (in dilution with boiled water 1:2 ratio) twice a day during 5-7 days; chewing 1 pill of a probiotic action “BioGaia ProDentis” twice a day during 2 weeks, and since the beginning of the second week of treatment – oral administration of the immune modulator “Imupret”, 25 drops 3 times a day during 3 weeks. Children from the comparative group was treated according to the common scheme: rinsing with antiseptic solution «Chlorhexidine bigluconate» 0,05% twice a day till the clinical effect of elimination of inflammatory signs, and «Rotocan» solution twice a day during 5-7 days; oral administration of the immune modulator «Echinacea» 5 drops (in dilution with boiled water 1:2 ratio) 3 times a day 30 minutes before meals during 3 weeks.

Results and their discussions. Biocoenosis of the oral cavity in children before the beginning of treatment were similar, and after the conducted first course of treatment microbiological status in both groups of the study improved immediately (Table 1).

The results of a quantitative assessment of the oral microflora after the conducted treatment demonstrated 69,42% ($p<0,05$) decrease of the general microbial number in children of the main subgroup, and in the children from the comparative group – 46,92% ($p<0,05$). The amount of gram-positive microorganisms in children from the main subgroup was found to be 73,69% less as compared with the results of treatment ($p<0,05$), gram-negative microorganisms – 46,43% less ($p<0,05$), and *Candida* fungi – 94,74% ($p<0,05$). These findings are twice as less in the group of comparison than those among the children from the main subgroup: gram-positive microorganisms – 54,77% less ($p<0,05$), gram-negative microorganisms – 36,00% ($p<0,05$), and *Candida* fungi – 42,86% ($p<0,05$). Comparison of findings between the main subgroup and the group of comparison determined 39,53% as much increased general amount of microorganisms, 47,37% - gram-positive, 6,25% – gram-negative and 87,50% - *Candida* fungi ($p_1<0,05$).

In addition to changes of the qualitative content of microorganisms found during inoculations from the oral cavity the rate of their growth changed as well. In 100% of cases among the children from the main subgroup very low colony growth was determined. Examination of inoculated cultures in the children from the group of comparison determined a moderate growth of the following microorganisms: streptococci (*Str. faecalis*, *Str. pyogenes*), staphylococci (*S. aureus*) and certain *Candida* (*C. albicans*). Scanty growth of colonies was found concerning other microorganisms.

Table 1. Changes in the amount of oral microorganisms in children suffering from chronic catarrhal gingivitis with comorbid diabetes mellitus in case of different methods of treatment, ($M \pm m$)

Indices before treatment		Main subgroup (n=30)		Group of comparison (n=24)	
		after treatment	before treatment	after treatment	before treatment
General amount of microorganism strains	abs.	85	26*	81°	43*°
	%	100	100	100	100
Amount of strains of gram-positive bacteria	abs.	38	10*	42°	19*°
	%	44,71	38,46	51,86	44,18
Amount of strains of gram-negative bacteria	abs.	28	15*	25°	16*
	%	32,94	57,69	30,86	37,21
Yeast-like fungi <i>Candida</i>	abs.	19	1*	14°	8*°
	%	22,35	3,85	17,28	18,61

notes: * – difference between the indices in the groups of observation before and after treatment is reliable ($p < 0,05$);
° – difference between the indices of the main and comparative groups in the dynamics of treatment is reliable ($p_1 < 0,05$)

Table 2. Changes of generic and specific content of microorganisms in the oral cavity of children suffering from chronic catarrhal gingivitis and comorbid diabetes mellitus in case of different methods of treatment, lg CFU/ml, ($M \pm m$)

kinds	Generic belonging	Specific belonging	Group of the study			
			main subgroup (n=30)		group of comparison (n=24)	
			before treatment	after treatment	before treatment	after treatment
resident	<i>Lactobacillus</i>	<i>L. salivarius</i>	0	1,00*	1,00°	1,00
	<i>Neisseria</i>	<i>N. oralis</i>	2,00±0,58	2,00±0,58	2,00±0,58	1,00*°
		<i>N. elongata</i>	2,00	2,00	2,00	1,00*°
	<i>Streptococcus</i>	<i>Str. anginosus</i>	3,00	1,0*	2,93±0,96	2,0
<i>Str. salivarius</i>		4,91±0,21	3,50±0,33	4,57±0,20	1,50±0,50*°	
<i>Str. faecalis</i>		3,75±0,48	1,0*	3,50±0,50	2,00*°	
opportunistic	<i>Staphylococcus</i>	<i>Str. pyogenes</i>	4,91±0,34	1,0*	4,67±0,33	2,00*°
		<i>S. epidermidis</i>	4,83±0,17	1,0*	4,75±0,25	2,00*°
		<i>S. aureus</i>	4,58±0,39	1,0*	4,50±0,17	2,00*°
	<i>Candida</i>	<i>C. albicans</i>	3,27±0,24	1,0*	3,00±0,19	2,0*°
		<i>C. tropicalis</i>	3,0±0,38	0	2,60±0,40	1,00*°
		<i>C. krusei</i>	2,00	0	1,00°	0
pathogenic	<i>Pseudomonas</i>	<i>P. aeruginosa</i>	3,33±0,33	0	3,33±0,33	2,0*
	<i>Escherichia</i>	<i>E. coli</i>	3,35±0,15	1,0*	3,23±0,17	1,22±0,15*°
	<i>Proteus</i>	<i>P. mirabilis</i>	3,50±0,50	1,0*	4,00	2,0*°
		<i>P. rettgeri</i>	2,00	0	2,00	0

notes: * – difference between the indices in the groups of observation before and after treatment is reliable ($p < 0,05$);
° – difference between the indices of the main and comparative groups is reliable ($p_1 < 0,05$)

After the course of treatment according to the suggested scheme, the contamination by associative opportunistic microflora in the oral cavity of children decreased reliably (Table 2).

We found 4,91 times less amount of *Str. pyogenes*, 4,58 times

less – *S. aureus* and 3,75 times – *Str. faecales*, 3,35 times *E. coli* and 3,27 times *C. albicans*. In children from the group of comparison contamination of the oral cavity with these microorganisms decreased as well: 2,65 times – *E. coli*, 2,34 times

Table 3. Changes of levels of mRNA expression of natural immunity in the buccal epithelium of children suffering from chronic catarrhal gingivitis with comorbid diabetes mellitus in case of different methods of treatment, (M±m)

Index	Before treatment		After treatment	
	Main subgroup (n=30)	Group of comparison (n=24)	Main subgroup (n=30)	Group of comparison (n=24)
TLR-2	90,07±3,41	85,83±5,02	40,35±4,91°	75,38±6,53°.*
TLR-4	75,55±7,33	79,57±8,44	41,22±9,47°	63,99±7,71°.*

notes: ° – difference between indices in the group of observation before and after treatment is reliable $p < 0,05$;

* – difference between indices of the main group and the group of comparison is reliable $p_1 < 0,05$.

Normalization according to the method $\Delta\Delta Ct$ with referent-agent GAPDH

Str. pyogenes, 2,25 times *S. aureus* and 75,00% – *Str. faecales*, 66,50% – *P. aeruginosa* and 50,00% – *C. albicans*.

Such representatives of biocoenosis of the oral cavity of children from the main subgroup as *Str. anginosus*, *P. aeruginosa*, *P. rettgeri*, *C. tropicalis* and *C. krusei* were not inoculated, and in children from the subgroup of comparison – only *P. zetteri* and *C. krusei* were found.

The state of natural immunity was also indicative of the improvement of protective mechanisms of the oral cavity in children from the main subgroup in the dynamics of treatment, therefore we have determined 2,23 times less level of mRNA TLR-2 expression ($p < 0,05$) in children from the main subgroup in the dynamics of treatment. This index was 46,48 % lower as much in comparison with the indices in children from the group of comparison ($p_1 < 0,05$), Table 3.

mRNA TLR-4 expression in children from both groups after treatment decreased reliably to (41,22±9,47) – in children from the main subgroup, and to (63,99±7,71) – in the subgroup of comparison, contrary to the indices before treatment. In children who were treated according to the common methods, mRNA TLR-4 expression was 35,58% higher than those from the values among the children from the main group ($p_1 < 0,05$).

Reduction of a relative normalized expression of TLR-2 and TLR-4 genes in children is associated with decreased number of gram-positive and gram-negative microorganisms in the oral cavity of children from the groups of observation.

Conclusions. Therefore, the conducted laboratory investigation is indicative of improvement of microbiological status and congenital mechanisms of immune protection in the oral cavity of children suffering from CCG and comorbid DM, when a complex of treatment is supplied with antiseptic, probiotic and immune modulator, which confirms greater efficacy of the suggested therapeutic-preventive complex than generally used scheme of treatment.

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SUMMARY

MICROBIOLOGICAL AND IMMUNOLOGICAL ASSESSMENT OF A COMPLEX OF THERAPEUTIC-PREVENTIVE MEASURES FOR CHRONIC CATARRHAL GINGIVITIS IN CHILDREN WITH DIABETES MELLITUS

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Dysbacteriosis of the oral cavity is known to complicate the course of chronic catarrhal gingivitis resulting from functional disorders of the macroorganism ecosystem, decreased amount of probiotic and increased amount of opportunistic and pathogenic microflora. And natural immune response is activated first in this case. It differentiates pathogens by TLR.

The aim - to enhance the efficacy of treatment of chronic catarrhal gingivitis in children suffering from type 1 diabetes mellitus by means of improving the methods of pharmacological correction on the basis of investigation of the indices of local natural immune response and microbiological properties of the disease.

We formed 2 group of the study. Children received basic insulin therapy. The treatment of chronic catarrhal gingivitis in children from the main group were suggested the antiseptic solution "Decasan"; pill of a probiotic action "BioGaia

ProDentis" and the immune modulator "Imupret". Children from the comparative group were treated according to the common scheme.

Oral microflora of children after treatment was decreased by 69,42% of general microbial number in children from the main group.

Natural immunity state was also indicative of the improved protective mechanisms of the oral cavity in children from the main subgroup in the dynamics of treatment: twice as less level of mRNA TLR-2 expression was found in the main subgroup and mRNA TLR-4 – 45,44%.

Therefore, the initiated course of treatment concerning chronic catarrhal gingivitis promoted a considerable improvement of the periodontal tissue state in children.

Keywords: gingivitis, microflora, toll receptors, antiseptic, probiotic, immune modulator.

РЕЗЮМЕ

МИКРОБИОЛОГИЧЕСКАЯ И ИММУНОЛОГИЧЕСКАЯ ОЦЕНКА КОМПЛЕКСА ЛЕЧЕБНО-ПРОФИЛАКТИЧЕСКИХ МЕРОПРИЯТИЙ У ДЕТЕЙ С ХРОНИЧЕСКИМ КАТАРАЛЬНЫМ ГИНГИВИТОМ И САХАРНЫМ ДИАБЕТОМ

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Целью исследования явилось повышение эффективности лечения хронического катарального гингивита у детей, больных сахарным диабетом типа 1, путем усовершенствования методов фармакологической коррекции, определения показателей местного врожденного иммунного ответа и микробиологических особенностей заболевания.

Обследованы 54 ребенка, больных сахарным диабетом типа 1 и гингивитом в возрасте 12 лет. В зависимости от проводимой терапии дети разделены на 2 группы: основная (n=30) и группа сравнения (n=24). Проведен микробиологический посев соскоба со слизистых оболочек ротовой полости с целью определения микрофлоры и уровня экспрессии мРНК показателей врожденного иммунитета в буккальном эпителии. Дети получали базисную терапию в виде инсулинотерапии по базисно-болюсной схеме. Лечение хронического катарального гингивита в обеих группах включало гигиеническое обучение индивидуального ухода за полостью рта, профессиональную

гигиену и санацию кариозных зубов. Детям основной группы предложено полоскание ротовой полости антисептическим раствором «Декасан», разжевывание 1 таблетки пробиотического действия «Биогайя ПроДентис», пероральное применение иммуномодулятора «Имупрет». Детям группы сравнения проводилось лечение хронического катарального гингивита по общепринятой схеме.

Микробный пейзаж полости рта детей после завершения лечебно-профилактических мероприятий характеризовался уменьшением общего микробного числа у детей основной группы до 69,42%, в группе сравнения - до 46,92%.

В динамике лечения состояние врожденного иммунитета указывает на улучшение защитных механизмов ротовой полости у детей основной группы, что подтверждается уменьшением уровня экспрессии мРНК TLR-2 вдвое у детей основной группы, в группе сравнения этот показатель стал меньше исходного уровня и составил 11,71%.

Экспрессия мРНК TLR-4 у детей обеих групп после ле-

чения достоверно уменьшилась: у детей основной группы - 45,44% а у детей в группе сравнения - 19,59%.

Полученные данные позволяют заключить, что проведенный нами курс лечебных мероприятий при хрониче-

ском катарального гингивите у детей основной группы способствовал существенному улучшению состояния тканей пародонта в сравнении с традиционным методом лечения.

რეზიუმე

ქრონიკულ კატარულ გინგივიტთან დაკავშირებული სამკურნალო-პროფილაქტიკური ღონისძიებების კომპლექსის მიკრობიოლოგიური და იმუნოლოგიური შეფასება შაქრიანი დიაბეტით ბავშვებში

ა.კოტელბანი, ჰ.მოროზი, ლ.გრინკევიჩი, დ.რომანიუკი, ტ.მურინიუკი

ბუკოვინის სახელმწიფო სამედიცინო უნივერსიტეტი, ბავშვთა სტომატოლოგიის კათედრა, ქირურგიის №1 კათედრა, ჩერნოვიცი, უკრაინა

კვლევის მიზანს წარმოადგენდა ქრონიკული კატარული გინგივიტის მკურნალობის ეფექტურობის ამაღლება ფარმაკოლოგიური კორექციის მეთოდების სრულყოფის, თანდაყოლილი ლოკალური იმუნური მახასიათებლების და დაავადების მიკრობიოლოგიური თავისებურებების განსაზღვრის გზით შაქრიანი დიაბეტი ტიპი-1-ით დაავადებულ ბავშვებში.

გამოკვლეულია 12 წლამდე ასაკის ქრონიკული კატარული გინგივიტი და შაქრიანი დიაბეტი ტიპი-1-ით დაავადებული 54 ბავშვი. ჩატარებული თერაპიის მიხედვით ბავშვები დაიყო ორ ჯგუფად: ძირითადი (n=30) და შედარების (n=24). პირის ღრუს ლორწოვანი გარსის ანაფხეკი მიკრობიოლოგიურად დაითესა მიკრობული ფლორისა და ბუკალურ ეპითელიუმში თანდაყოლილი იმუნიტეტის რნმ-მაჩვენებლების ექსპრესიის დონის განსაზღვრისათვის. ბავშვები იღებდნენ ბაზისურ თერაპიას ინსულინოთერაპიის სახით ბაზისურ-ბოლუსური სქემით. ქრონიკული კატარული გინგივიტის მკურნალობა ორივე ჯგუფში მოიცავდა პირის ღრუს ინდივიდუალური ჰიგიენური მოვლის, პროფესიონალური ჰიგიენის და კარიესული კბილების სანაციის სწავლებას. ძირითადი ჯგუფის ბავშვებმა მიიღეს შეთავაზება პირის ღრუში ანტისეპტიკური ხსნარის "დაკასანის" გამოვლენის, პრობიოტური მოქმედების ტაბლეტის (1 ც.) "ბიოგაია პროდენტის" დაღუჭვის და იმუნომოდულატორ "იმუპრეტი"-ს პერორალური გამოყენების შესახებ. შედარების ჯგუფის ბავშვებში ქრონი-

კული კატარული გინგივიტის მკურნალობა ტარდებოდა მიღებული სქემით.

სამკურნალო-პროფილაქტიკური ღონისძიებების დასრულების შემდეგ ბავშვების პირის ღრუს მიკრობული პეიზაჟი ხასიათდებოდა მიკრობების საერთო რაოდენობის შემცირებით ძირითად ჯგუფში 69,42%-ით, შედარების ჯგუფში კი - 46,92%-ით.

თანდაყოლილი იმუნიტეტის მდგომარეობა მკურნალობის დინამიკაში მიუთითებს პირის ღრუს დაცვითი მექანიზმების გაუმჯობესებაზე ძირითადი ჯგუფის ბავშვებში, რაც დასტურდება ციტოკინური კასკადის რეაქციის მაინცირებელი და პაროდონტის ქსოვილში ანთებითი პროცესების განვითარების განმაპირობებელი Toll-მსგავსი რეცეპტორების - TRL-2-ის რნმ-ის ექსპრესიის დონის ორჯერ შემცირებით ძირითადი ჯგუფის ბავშვებში; შედარების ჯგუფში ეს მახასიათებელი საწყის დონესთან შედარებით შემცირდა და შეადგინა 11,71%.

TRL-4-ის რნმ-ის ექსპრესია ორივე ჯგუფის ბავშვებში სარწმუნოდ შემცირდა: ძირითადი ჯგუფის ბავშვებში - 45,44%, შედარების ჯგუფში - 19,59%.

მიღებული შედეგები იძლევა საფუძველს დასკვნისათვის, რომ ავტორების მიერ ჩატარებულმა სამკურნალო ღონისძიებების კურსმა ქრონიკული კატარული გინგივიტის მქონე ძირითადი ჯგუფის ბავშვებში ხელი შეუწყო პაროდონტის ქსოვილების მდგომარეობის მნიშვნელოვან გაუმჯობესებას, მკურნალობის ტრადიციულ მეთოდებთან შედარებით.

SURVEY OF PRACTICES, KNOWLEDGE AND ATTITUDE CONCERNING ANTIBIOTICS AND ANTIMICROBIAL RESISTANCE AMONG MEDICAL UNIVERSITY STUDENTS

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Antimicrobial resistance (AMR) has become a global problem and is considered an emerging threat to public health worldwide [2,5,6,8,19]. It prevents successful treatment of many infections. In 2001 WHO provided a program to slow the emergence of AMR and reduce the number of resistant microorganisms and in 2012 it published the Options for Action [19]. According to WHO, “AMR is a high priority issue to be resolved by collective global action” [25]. Antibiotics are very important part of care in multiple fields of medicine and increase of resistant microorganisms raises the costs for effective treatment and endangers life of the patients in general causing higher morbidity and mortality rates.

Some of the main reasons of AMR are availability of antibiotics over the counter, self medication, inappropriate use and non-compliance, overprescription by the physicians, lack of knowledge among population and specialists, poor quality of the drugs, inappropriate storage conditions, the use of antibiotics in animal and agricultural industry, release of a large amount of antibiotics in wastewater, absence or lack of the antimicrobial resistance control strategies, etc. [3,9,10,11,19,26].

There are several ways to improve the situation and one of them is education of the health professionals on effect of the misuse of antibiotics and education of the general population on importance of avoiding self-medication and misuse of antimicrobials [3].

In order to tackle this problem in the most efficient way, it is necessary first of all to research the current situation in the country, i.e. among general population and medical professionals, including students. Appropriate studies are quite rare and none exist regarding the situation in Georgia.

The aim of our study was to assess knowledge, attitude and practice (KAP) regarding antimicrobial resistance and antibiotics among medical students in Georgia.

Material and methods. *Questionnaire development.* Literature search and analysis of the similar studies was performed [1,4,11,13,14,16,17,18,22]. Based on them potential questions were determined. The specialists in the field (general practitioner, pediatrician, infectionist) were asked for the opinion on these questions. The KAP questionnaire was developed in English and piloted in a group of foreign students of the International Faculty of Medicine. The participants of the pilot study were

excluded from the main study. They were asked to provide their opinion regarding relevance and difficulty of the questions. Following the discussion and taking into account their comments, the final version was developed. It contains 50 questions: 3 demographics, 15 knowledge, 16 attitude, 16 practice. The English version was translated into Georgian and approved by the Department of Epidemiology and Biostatistics.

Sampling. Sampling frame consisted of students of the 4th year of the Tbilisi State Medical University. Random cluster sampling was used with confidence interval/margin of error 10 and confidence level 95%. Total number of participants was 212: 31.13% from the International Faculty of Medicine (English) (n=66), 45.75% from the Faculty of Medicine (medicalGEO) (n=97), and 23.11% from the Faculty of Public Health (publicHEALTH) (n=49). Mean age 22.95 (sd=0.52); 50.0% male (n=106) and 47.64% female (n=101), 5 persons have not responded to this question.

Survey. Cross-sectional study was performed. The survey was anonymous and self-administered. Identical English and Georgian questionnaires were used. The survey was done in groups and before starting, the respondents were informed of the aim of the study and explained its significance. Each questionnaire had an introductory paragraph which contained explanation of the main terms, instruction on how to fill the questionnaire, note that this was voluntary and expression of gratitude for the spent time. Principles of Declaration of Helsinki were followed.

Data analysis. The electronic replica of the questionnaire was prepared in Epidata 3.1. It gave the possibility to use the checks option to introduce the data entry error control. All questionnaires were given the unique number. After manual check all questionnaires were entered into Epidata and then exported to Stata 14.0. Data analysis was done in Stata 14.0. Descriptive statistics was used to generate frequencies, percentages and proportions. Where relevant, the Chi-square test was used to determine any statistical significance.

Results and their discussion. Knowledge

79.25% think that antibiotics can cure bacterial infections, 16.51% think that no, and 4.25% do not know. 33.02% think that antibiotics can cure viral infections, 57.08% think that no, and 9.91% do not know. There was a statistically significant difference by faculties ($p < 0.0001$) (Figs. 1, 2).

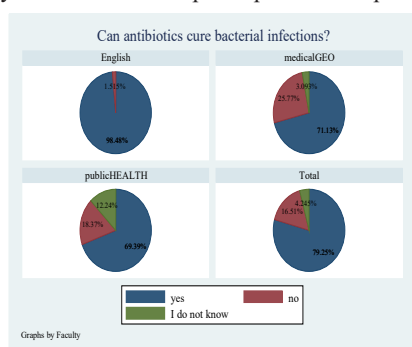


Fig. 1. Percentage of responses to the question: “Can antibiotics cure bacterial infections?”

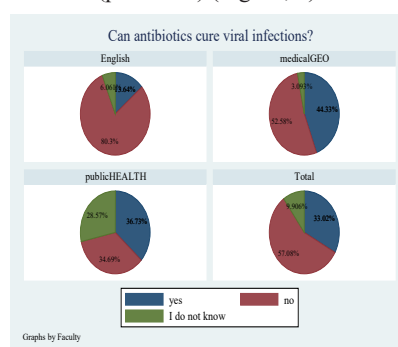


Fig. 2. Percentage of responses to the question: “Can antibiotics cure viral infections?”

Equal number of the students (46.23%) think that use of antibiotics will speed up the recovery of colds and 7.55% do not know. There is no statistically significant difference by gender or faculties. 34.43% think that bacteria cause common cold and

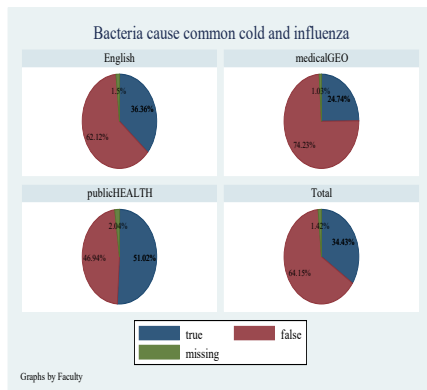


Fig. 3. Percentage of agreement to the statement: "Bacteria cause common cold and influenza"

56.60% of the respondents agree that the unsystemic use of antimicrobials leads to the emergence of resistance, but 43.40% do not. There is a statistically significant difference by faculties ($p < 0.0001$). 13.68% do not agree with the meaning of antimicrobial resistance. There is a statistically significant difference by faculties ($p = 0.021$). 83.96% recognize that Antibiotic Resistance is an important and serious global public health issue. There is a statistically significant difference by faculties ($p = 0.013$).

70.28% think that frequent use of antibiotics will decrease efficacy of treatment when using the antibiotic again, but 16.51% do not think so and 13.21% do not know. There is no statistically significant difference by gender or faculties. 44.34% think that the efficacy is better if the antibiotics are newer and more costly, though 32.08% do not think so and 22.64% do not know. There is a statistically significant difference both by gender ($p = 0.001$) and by faculties ($p = 0.007$). 25.00% agree that unsystemic and disorganized use of antibiotics might contribute to treatment efficacy. There is a statistically significant difference by faculties ($p < 0.0001$). 77.36% agree that unsystemic use of antibiotics might prolong the disease. There is a statistically significant difference by faculties ($p < 0.0001$). 24.53% erroneously agree that due to unsystemic use of antibiotics the additional costs for the patient might decrease. There is a statistically significant difference by faculties ($p < 0.0001$). 83.49% agree that if taken too frequently the antibiotics will not work in the future. There is a statistically significant difference by faculties ($p < 0.0001$).

Attitude

85.85% agree that there is abuse (taken too frequently or when there is no necessity) of antibiotics at present. 83.96% agree that antibiotics resistance has become a problem. 75.00% agree that the abuse of antibiotics has become the main cause leading to bacterial resistance. 60.38% consider that antibiotic resistance affects them and their family's health. For these statements there is no statistically significant difference by gender or faculties.

93.40% agree that it is necessary to get more education about antibiotics and 79.25% think that it is necessary to establish course on 'rational use of antibiotics'. There is a statistically significant difference by faculties ($p < 0.0001$).

Frequency of responses to different statements regarding attitude to AMR and antibiotics are presented in Table 1.

influenza. There is a statistically significant difference both by gender ($p = 0.01$) and by faculty ($p = 0.03$) (Fig. 3). 83.02% have heard of antibiotic resistance. There is a statistically significant difference by faculties ($p < 0.0001$) (Fig. 4).

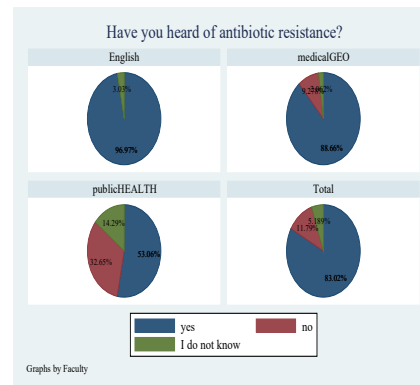


Fig. 4. Percentage of responses to the question: "Have you heard of antibiotic resistance?"

Practice

The students' behaviour of using antibiotics is illustrated in the Table 2.

This is the first nationwide survey to explore the knowledge, attitude and behaviour regarding antibiotic use among students in Georgia. Generally the obtained results were corresponding to the findings from the studies performed in other countries, though there were some differences as well.

74.94% of our respondents are aware that antibiotics cure bacterial infections, so are around 92% of medical students in China [12,13], 93.3% in UAE [14], 95.2% in Italy [21]. 98% in India [11], 70.4% in Jordan [23]. 57.08% think that antibiotics can NOT treat viral infections, the same think 64.5% of medical students in China [13], 65.8% in UAE [14], 83.2% in Italy [21], 55% in India [11], 71.9% in Jordan [23]. 64.15% know that bacteria do NOT cause common cold and influenza, the same think around 76% of medical students in India [4,16] and 71.9% in Jordan [23]. 83.02% of our respondents have heard of antibiotics resistance, 94.7% of medical students in China [13], 84.5% in UAE [14] and 93% in India [11]. 84.28% agree with AMR definition, 93.9% in medical students in Italy [21]. 94% of Sri Lanka pharmacy students were aware of the terms antibiotic resistance and 76% of antimicrobial resistance [20]. 70.28% think that frequent use of antibiotics will decrease efficacy of treatment when using the antibiotic again, likewise think 87.9% of medical students in China [13], 82.4% in Ethiopia [22] and 97% in India [11]. 32.08% do NOT think that the efficacy is better if the antibiotics are newer and more expensive; the same think 81.5% of medical students in China [13], 72.5% in UAE [14] and 60% in India [11]. 56.60% agree that the unsystematic use of antibiotics leads to the resistance. The same think 97% of medical students in US [1], 94.1% in Ethiopia [seid], 93.9% in Italy [21], 95.1% in Malaysia [15]. 77.36% of our respondents think that this might prolong the disease, the same think 84.3% in Jordan [23]. 46.23% think that use of antibiotics can NOT speed up the recovery of colds and flu. The same think 27.4% of medical students in China [13], 34.2% in UAE [14], 35% in Ethiopia [22], 72% in India [11].

85.85% of TSMU students agree that there is abuse of antibiotics at present; the same think around 85% of medical students in China [12,13], 91.3% in UAE [14], 87.5% in Congo [24],

Table 1. Attitudes towards antibiotics and AMR

<i>in %</i>	completely agree	somewhat agree	undecided	somewhat disagree	completely disagree
Antibiotics are safe drugs, hence they can be commonly used*	14.15	26.42	12.74	25.00	21.70
Skipping one or two doses does not cause development of antibiotic resistance	21.23	27.83	15.09	19.81	15.57
Adverse effects of antimicrobials are reduced by using more than one antibiotic at a time**	6.60	22.17	24.53	18.40	27.83
Unnecessary use of antimicrobials shortens the duration of illness*	4.25	17.45	16.04	14.15	47.17
When you have a cough and sore throat, use of antimicrobials will contribute to emergence of resistant strains	14.15	36.32	21.70	14.62	13.21
When I have a cold, I should take antibiotics to prevent getting a more serious illness*	16.98	21.23	8.96	16.98	34.43
When I get fever, antibiotics help me to get better more quickly***	18.87	25.94	14.62	25.47	15.09
Whenever I take an antibiotic, I contribute to the development of antibiotic resistance	18.40	29.72	21.70	19.34	9.91
AMR is an important and serious public health issue facing the World**	54.25	25.94	11.79	4.72	2.83
AMR is an important and serious public health issue in our Country	50.47	24.06	16.51	4.25	4.25

after combining the answers into 3 categories: agree, undecided and disagree,

* there is a statistically significant difference by faculties when ($p < 0.0001$); ** there is a statistically significant difference by faculties ($p = 0.005$); *** there is a statistically significant difference by faculties ($p = 0.002$)

Table 2. Practice of using antibiotics

<i>in %</i>	always	often	sometimes	never
The doctor prescribes a course of antibiotic for you. After taking 2–3 doses you start feeling better.				
... Do you stop taking the further treatment?*	12.74	18.87	25.94	42.45
... Do you save the remaining antibiotics for the next time you get sick?*	12.74	15.09	28.77	43.40
... Do you discard (throw away) the remaining, leftover medication?***	11.79	8.02	29.72	50.47
... Do you give the leftover antibiotics to your friend if he/she gets sick?****	8.49	10.85	26.42	53.77
Do you consult a doctor before starting an antibiotic?	31.60	30.19	33.02	4.72
Do you check the expiry date of the antibiotic before using it?*****	56.60	17.45	18.40	6.60
Do you use antibiotics when you ...				
... have Fever?	12.74	26.42	39.62	20.75
... have Common cold?*****	9.43	14.62	28.30	47.17
... have Acute bronchitis?*	18.87	26.42	35.38	16.98
... are Coughing up yellow/green sputum?*****	23.58	23.11	30.66	22.64
... have Sore throat?*****	9.91	20.28	34.91	33.49
... have Cough with fever?	17.45	25.47	39.15	16.98
... have Congested nose with headache?*****	10.85	9.91	21.70	56.13
... are Coughing up white sputum?*	12.26	11.79	34.43	41.04
... have Cough lasting 2 weeks or more?*****	19.34	20.75	35.38	23.11
Do you ask a doctor to prescribe antibiotics when you catch a common cold?*****	8.02	11.79	29.25	50.94

After combining the answers into 2 categories: yes and no,

* there is a statistically significant difference by faculties when ($p < 0.0001$); ** there is a statistically significant difference by faculties ($p = 0.005$); *** there is a statistically significant difference by faculties ($p = 0.001$); **** there is a statistically significant difference by faculties ($p < 0.05$)

93% in India [11]. 83.96% of respondents agree that antibiotics resistance has become a problem likewise 82.84% of medical students in China [13], 47.8% in UAE [14], 94.0% in France [7], 70.6% in Ethiopia [22], 99% in India [11] and majority in Congo [24]. 75% agree that the abuse of antibiotics has become a main cause leading to AMR, the same think 83.88% of medical students in China [huang], 79.2% in UAE [jairoun] and 95% in India [11]. 60.38% think that AMR affects them and their family's health in comparison to 81.9% of medical students in China [13], 70.8% in UAE [jairoun], 90.7% in Ethiopia [22], 92% in India [11]. 93.40% agree that more education on antibiotics is necessary likewise 89.2% of medical students in China [13], 97.2% in UAE [14], 94% in India [11]; 90.0% of Miami students would like to get more education on antimicrobials and 79% on antimicrobial resistance [1].

Comparison of practical behaviour regarding antibiotics between our respondents and medical students from China, UAE and India is given on Fig. 5.

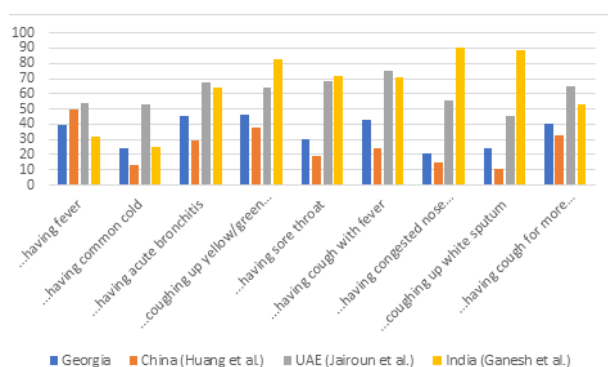


Fig. 5. Frequency of using antibiotics always and often (in %)

31.61% of the students in Georgia always and often stop taking antibiotics if they start feeling better. Same behaviour is seen in 34.7% of medical students in UAE [14], 5.7% in Malaysia [15], 15.2% in Italy [21], 61.2% in Jordan [23].

43.40% never keep the remaining antibiotics and 19.81% always and often discard them. Keeping leftover antibiotics is not a good practice for 96.7% of medical students in Malaysia [15]. 17.7% use leftover antibiotics and 62.01% have them at home among medical students in Italy [21]. 73.1% of medical students in Jordan also keep them at home [23].

There was statistically significant difference in responses to some questions by faculty and this is also in line with the international findings [13]. It is understandable that without a specially dedicated course on AMR the background of the Medicine students gives them more general knowledge on antibiotics and antimicrobial resistance than public health students. But this should be improved, because AMR is a global health problem to be handled not only by the clinical staff but by the public health professionals as well.

Limitation of the study is that we have not introduced questions to assess the knowledge of the participants regarding the causes of AMR. Also it would have been interesting to compare medical students with non-medical students. Another interesting issue that can be explored in the future research is adding additional variables such as parental background, academic achievement level, economic status, etc. in order to establish possible correlations.

Conclusion.

Obtained results are in line with the international findings. There was seen a statistically significant difference between

public health and medical faculties (both English and Georgian) in responses on knowledge. In the attitude and practical behaviour sections all three groups provided similar results, only some questions were provided significantly different responses by faculties. There was no statistically significant difference by gender. Difference in responses, especially on knowledge can be explained by the background of different faculties. But this problem is important not only for future clinicians but for the public health specialists as well and it is advisable to introduce additional training in this direction.

Acknowledgements: We would like to thank the students who participated in the research for their input in study of this important issue.

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SUMMARY

SURVEY OF PRACTICES, KNOWLEDGE AND ATTITUDE CONCERNING ANTIBIOTICS AND ANTIMICROBIAL RESISTANCE AMONG MEDICAL UNIVERSITY STUDENTS

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Antimicrobial resistance (AMR) has become a global problem and is considered an emerging threat to public health worldwide.

Aim - to assess knowledge, attitude and practice (KAP) regarding antimicrobial resistance and antibiotics among medical students in Georgia.

KAP questionnaire containing 50 questions was developed in English and Georgian. Sampling frame consisted of students of the 4th year of the Tbilisi State Medical University (TSMU). Random cluster sampling was used and total number of participants was 212 (31.13% - International Faculty of Medicine, 45.75% - Faculty of Medicine, 23.11% - Faculty of Public Health). Mean age 22.95 (sd=0.52) with almost equal gender distribution. Cross-sectional study was performed. The survey was anonymous and self-administered. Principles of Declaration of Helsinki were followed. Data analysis was done in Stata 14.0.

79.25% think that antibiotics can cure bacterial infections, 57.08% think that antibiotics can not cure viral infections (statistically significant difference by faculties $p < 0.0001$). 34.43% think that bacteria cause common cold and influenza (by gender $p = 0.01$ and by faculties $p = 0.03$). 83.02% have heard of antibiotic resistance (by faculties $p < 0.0001$). 83.96% agree that antibiotic resistance has become a problem. 75.00% agree that the abuse of antibiotics has become the main cause leading to bacterial resistance. 60.38% consider that antibiotic resistance affects them and their family's health. 93.40% agree that it is necessary to get more education about antibiotics. 31.61% of the students in Georgia always and often stop taking antibiotics if they start feeling better. 43.40% never keep the remaining antibiotics.

Obtained results are in line with the international findings. There was seen a statistically significant difference between public health and medical faculties (both English and Georgian) in responses on knowledge. In the attitude and practical behaviour sections all three groups provided similar results, only some questions were provided significantly different responses by faculties.

Keywords: AMR, KAP, students, antibiotics, antimicrobials, resistance, survey

РЕЗЮМЕ

ОЦЕНКА ЗНАНИЙ, ОТНОШЕНИЯ И ПРАКТИЧЕСКОГО ПОВЕДЕНИЯ СТУДЕНТОВ МЕДИЦИНСКОГО ВУЗА ПО ИСПОЛЬЗОВАНИЮ АНТИБИОТИКОВ И ИХ ПРОТИВОМИКРОБНОЙ РЕЗИСТЕНТНОСТИ

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Противомикробная резистентность является всемирной проблемой и представляет угрозу для здравоохранения во всем мире.

Цель исследования - оценка знаний, отношения и практического поведения студентов медицинского университета по использованию антибиотиков и их противомикробной резистентности.

Опросник знаний, отношения и практического поведения (ЗОП), содержащий 50 вопросов разработан на английском и грузинском языках. В исследовании принимали участие студенты IV курса Тбилисского государственного медицинского университета. В исследовании использован метод

случайной гнездовой выборки и общее количество участников составило 212 (31,13% - международный факультет медицины, 45,75% - факультет медицины, 23,11% - факультет здравоохранения; почти равномерное разделение по полу). Средний возраст 22,95 (sd=0,52). Проведено поперечное исследование. Опрос был анонимным и выполнялся самостоятельно, соблюдены принципы Хельсинкской декларации. Анализ данных проведен в программе Stata 14.0.

Согласно данным опросника, 79,25% опрошенных студентов считают, что антибиотики лечат бактериальные инфекции, 57,08% - антибиотики не лечат вирусные инфекции (статистически значимая разница по факультетам $p < 0,0001$). 34,43% - бактерии вызывают простуду и грипп (по полу $p = 0,01$, по факультетам $p = 0,03$). 83,02% осведомлены об антибиотикорезистентности (по факультетам $p < 0,0001$). 83,96% считают, что антибиотикорезистентность является проблемой, а 75,00% - что злоупотребление антибиотиками - главная причина, вызывающая бактериальную резистентность. По мнению 60,38% опрошенных студентов, антибиотикорезистентность наносит ущерб здоровью. 93,40% студентам необходимо получить больше знаний об антибиотиках. 31,61% опрошенных студентов всегда и часто прекращают прием антибиотиков, если чувствуют себя лучше.

На основании анализа полученных данных делается вывод, что результаты проведенного исследования аналогичны международным. Выявлена статистически значимая разница между ответами студентов факультетов здравоохранения и медицины (англоязычный и грузиноязычный). В разделах по отношению и практическому поведению все три группы показали схожие результаты, лишь на некоторые вопросы представлены ответы, значительно различающиеся по факультетам. Статистически значимой разницы в ответах студентов женского и мужского пола не выявлено.

რეზიუმე

სამედიცინო უნივერსიტეტის სტუდენტების ცოდნის, დამოკიდებულების და პრაქტიკული ქცევის შეფასება ანტიბიოტიკების გამოყენებისა და მათი ანტიმიკრობული რეზისტენტობის შესახებ

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თბილისის სახელმწიფო სამედიცინო უნივერსიტეტი, ¹ეპიდემიოლოგიის და ბიოსტატისტიკის დეპარტამენტი; ²ბავშვთა და მოზარდთა მედიცინის დეპარტამენტი, საქართველო

ანტიმიკრობული რეზისტენტობა (AMR) წარმოადგენს გლობალურ პრობლემას და დიდ საფრთ-

ხეს საზოგადოების ჯანმრთელობისათვის მოელი მსოფლიოს მასშტაბით.

კვლევის მიზანი - სამედიცინო უნივერსიტეტის სტუდენტების ანტიბიოტიკების გამოყენებისა და მათი ანტიმიკრობული რეზისტენტობის შესახებ ცოდნის, დამოკიდებულების და პრაქტიკული ქცევის შეფასება.

აღნიშნული ინფორმაციის მისაღებად გამოყენებული იყო ცოდნის, დამოკიდებულების და პრაქტიკული ქცევის (კვპ) ქართულ და ინგლისურენოვანი კითხვარი, რომელიც შედგებოდა 50 შეკითხვისაგან. კვლევაში მონაწილეობდა თბილისის სახელმწიფო სამედიცინო უნივერსიტეტის IV კურსის 212 სტუდენტი (31,13% - მედიცინის საერთაშორისო ფაკულტეტი, 45,75% - მედიცინის ფაკულტეტი, 23,11% - ჯანდაცვის ფაკულტეტი; სქესის მიხედვით თითქმის თანაბარი განაწილება), საშუალო ასაკი $22,95 \pm 0,52$ წ. ჩატარდა ერთმომენტიანი ანონიმური კვლევა, რომელიც გულისხმობდა კითხვარის დამოუკიდებლად შევსებას. დაცული იყო ჰელსინკის დეკლარაციის პრინციპები. მიღებული მონაცემები დამუშავდა პროგრამით „Stata 14.0“.

მიღებული მონაცემების ანალიზის შედეგად გამოვლინდა, რომ სტუდენტების უმეტესობის ნაწილის აზრით (79,25%), ანტიბიოტიკებით შესაძლებელია ბაქტერიული ინფექციების განკურნება; 57,08% კი, რომ ანტიბიოტიკები არ კურნავს ვირუსულ ინფექციებს (სტატისტიკურად მნიშვნელოვანი განსხვავებაა ფაკულტეტებს შორის $p < 0,0001$). 34,43% თვლის, რომ გაცივებას და გრიპს იწვევს ბაქტერია (სქესის მიხედვით $p = 0,01$ და ფაკულტეტების მიხედვით $p = 0,03$). 83,02%-ს გაცნობიერებულია ანტიბიოტიკორეზისტენტობის შესახებ (ფაკულტეტების მიხედვით $p < 0,0001$). 83,96% ეთანხმება მოსაზრებას, რომ ანტიბიოტიკორეზისტენტობა სადღეისოდ წარმოადგენს პრობლემას, 75,00%-ს მიაჩნია, რომ ანტიბიოტიკების ბოროტად გამოყენება არის AMR-ის მთავარი გამომწვევი მიზეზი. 60,38% მიიჩნევს, რომ AMR გავლენას ახდენს მათ და მათი ოჯახის წევრების ჯანმრთელობაზე. 93,40% თვლის, რომ საჭიროა მეტი ცოდნის მიღება ანტიბიოტიკების გამოყენების და გვერდითი მოვლენების შესახებ. გამოკითხული სტუდენტების 31,61% ყოველთვის და ხშირად წყვეტს ანტიბიოტიკების მიღებას, თუ თავს უკეთ გრძობს. 43,40% არასოდეს ინახავს დარჩენილ ანტიბიოტიკებს.

მიღებული შედეგები შეესაბამება საერთაშორისო შედეგებს. სტატისტიკურად მნიშვნელოვანი განსხვავება გამოვლინდა საზოგადოებრივი ჯანდაცვის და მედიცინის ფაკულტეტების სტუდენტების პასუხებში. დამოკიდებულების და პრაქტიკული ქცევის ნაწილებში სამივე ჯგუფმა მსგავსი შედეგები აჩვენა. სტატისტიკურად მნიშვნელოვანი განსხვავება პასუხებში სქესის მიხედვით არ გამოვლინდა.

PREVENTION OF CEREBROVASCULAR MICROEMBOLIZATION DURING AORTA-CORONARY BYPASS UNDER CONDITIONS OF ARTIFICIAL BLOOD CIRCULATION

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Coronary heart disease (CHD) is one of the leading causes of disability and mortality worldwide [6,7]. Despite significant progress in the field of CHD pharmacotherapy, it is not always effective to achieve the desired results [8], which is the indication for use of surgical methods of revascularization. Coronary bypass surgery (CABG) is one of the most common surgical methods for the treatment of coronary artery disease, and is often performed under conditions of artificial blood circulation (ABC).

However, such operations are associated with the risk of damage to the central nervous system (CNS), the development of neurological and neuropsychological disorders of varying severity related to the features of the surgical and perfusion techniques [1,5,10].

A special place among these disorders is occupied by postoperative cognitive dysfunction (POCD) [4,7,10,18], which is a marker of poor quality of life, financial dependence of the patient on relatives and the state social services, and an unfavorable prognosis for survival [2,19,22].

In the recommendations of the American Heart Association (AHA) and the American College of Cardiology (ACC) in 2014, cerebral complications of cardiac surgery are divided into 2 types: [16].

Cerebral injuries of type 1 are associated with stroke death, fatal stroke, or transient ischemic attack, while delirium and postoperative cognitive dysfunction (POCD) are classified as cerebral lesions of the type 2 [2].

The two most significant clinical neurological complications of CABG are stroke and POCD. The stroke incidence among the patients undergoing CABG is 1.6% [2]. However, the quantitative determination of POCD is more complex. According to various authors, the incidence of encephalopathy in the postoperative period reaches 40-90% [9,12,18].

POCD is a decrease in cognitive function after surgery and anesthesia compared with the preoperative baseline [1,2,9,14]. POCD can manifest itself in various cognitive areas, such as attention, memory, learning, visual spatial, motor skills, and executive functions. It may also be accompanied by behavioral changes.

The up-to-date concept of POCD consists in the development of disorders of higher cortical functions in the early postoperative period, and their preservation later [4,7,21].

Clinically, POCD can be confirmed using neuropsychological testing conducted immediately after surgery and in the late postoperative period, compared with baseline studies that had been performed before surgery. However, currently there is no international agreement on generally accepted criteria for making a diagnosis of POCD; its structure is not clear; there are no uniform approaches to conducting neuropsychological testing [22].

Numerous studies indicate that the pathogenesis of POCD is multifaceted [2,9,10,23]. At the same time, discussions and search for the most important factors for the development of POCD in cardiac surgery patients are still ongoing.

The issues of prevention of brain function impairment during cardiac surgery performed under cardiopulmonary bypass are still not fully understood and largely unsolved [12].

In the modern scientific literature, the following mechanisms of brain damage in cardiac surgery patients operated under conditions of artificial blood circulation (IC) are being discussed [1,4,10,11,13,18,20,24,25]:

- intraoperative microembolization of cerebral arteries;
- systemic hypoperfusion within the central nervous system with distortion of the autoregulation of the cerebral blood flow;
- systemic and cerebral inflammatory response;
- cerebral edema;
- dysfunction of the blood-brain barrier;
- contact activation of blood cells in the course of the skin;
- metabolic disorders;
- pharmacological effects of anesthetics;
- other surgical and individual factors

It is important to note that the role of each of the above pathogenetic factors of perioperative damage to the brain is discussed by modern researchers, and the concept of etiopathogenesis of disturbance of neurological functions in cardiosurgical patients in the postoperative period has not yet been formulated.

Intraoperative microemboli are registered in the overwhelming majority of cardiosurgical patients. Reliable relationship between the number of cerebral microemboli, on the one hand, and postoperative cognitive impairments, on the other hand, was demonstrated in a significant number of neuropsychological studies [4,7,19].

A number of studies have demonstrated the relationship between the amount of cerebral arterial microembolization and the degree of brain damage in operations with MS [11,13,18,20,24,25]. However, the available literature does not adequately cover the methods of prophylaxis of gas embolism and the possibility of using systemic vasodilators [3] to reduce the risk of cerebral arterial microembolization.

We were of the opinion that application of high doses of nitroglycerin at the end of artificial blood circulation against the background of high volume perfusion rates could reduce the severity of brain damage in the postoperative period.

The aim of the study - to investigate the relationship between cerebral microembolization and the development of postoperative cognitive impairment in patients after coronary artery bypass grafting with and without prophylactic administration of nitroglycerin at the end of artificial blood circulation.

Material and methods. The study included two groups of patients: control and experimental. The total number of patients was 72 (43 male and 29 female), who underwent CABG procedure due to myocardial infarction. The control group consisted of 34 patients, the study group - 38 patients.

The average age of patients was 67.3±5.2 years old. The average body mass index was 27.4±5.2 kg/m². The average duration of the operation is 269.4±7.8 minutes, the artificial blood circulation - 145±4.5 min.

Depending on the functional class (FC), all patients were distributed as follows: II FC - 9 patients (12.5%), III FC - 51 patients (70.8%), IV FC and unstable angina pectoris - 6 patients (16.7%)

There were 49 (68%) patients with the history of one myocardial infarction (MI), and 8 (11.1%) patients with the history of 2 MI. Seven (22,2%) patients with coronary heart disease (CHD) indicated no prior history.

The left ventricular ejection fraction (FV) above 40% was recorded in 58 (80.6%) patients, and below 40% - in 14 (19.4%) patients.

Carotid duplex scan conducted at the preoperative stage on all the patients, did not detect any hemodynamically significant stenoses within the major neck area arteries [15,24].

All patients were operated under general anesthesia with sevoflurane (1.5-2Mac). Introductory anesthesia included propofol (1.5±0.3 mg/kg) and fentanyl (1 µg/kg). Myorelaxation was facilitated by use of pipecuronium bromide (0.07-0.08 mg/kg); further analgesia - fentanyl (21.5±3.4 µg / kg for the full duration of surgery).

Artificial ventilation of the lungs was carried out by the air-oxygen mixture (FiO₂=50%), under the arterial blood gas monitoring (the average value of pCO₂ of the arterial blood was 35.3±2.4 mm Hg).

The medical clearance examination of the patients was performed 3 days prior to the operation and, together with the generally accepted methods, included transtemporal dopplerography by the Siemens apparatus (Germany).

In the course of all operations the standard protocol of anesthesia, surgical technique and artificial blood circulation was used.

Peripheral vasodilator (nitroglycerin) at the dose of 8-10 µg/kg·min were administered to patients of the study group at the end of the artificial blood circulation for 9-10 minutes against the background of high (120-130% of the estimated) volume perfusion rate.

Assessment of the cognitive status of patients was performed using verbal and non-verbal tests (one day before the surgery, and on the fifth day after).

1. MOCA - Montreal Scale for Assessing Cognitive Functions [6,17] – is a set of tests designed to quickly assess cognitive impairment. The test evaluates memory, “frontal” functions (visual-constructive skills, speed of speech, abstraction, etc...), nominative function of language, visual spatial praxis. The average duration of evaluation in elderly patients was 15-20 minutes. In the postoperative period, alternate test variants were used for evaluation on the MCA scale (alternative words were used in the test for memorization, and different figure in the copy test);

2. MMSE - short scale of assessment of mental status [6] - the technique is widely used to diagnose dementia. Orientations, memory, attention, speech, executive functions and constructive praxis are studied. Duration of the survey in the elderly usually did not exceed 15 minutes.

3. Comprehensive study of various cognitive functions: Trail-making test, Grooved Pageboard, Tests for fine hands, learning 10 words for AR Luria, Wexler’s test, Schultz tables.

The criterion for diagnosis of POPs was to reduce test results by 10% or more, with a full study in two, or more tests, and a decrease of 2 points or more in the MoCA and MMSE tests.

Intraoperative monitoring of cerebral blood flow was performed on the device “Angiodin 2K” (“Bios”, Russia). Measurements were made by transtemporal access according to the standard method [15]. The baseline evaluation of linear blood flow rate was performed the night before the surgery, and subsequently intraoperatively, starting with the stage of anesthesia induction. In this case ultrasonic sensors of linear format with the frequency range of 5-10 MHz were used.

Statistical processing was performed using dispersion and correlation analysis methods using Statistica 10.0 software (Dell StatSoft, USA).

Results and their discussion. The obtained data show that, regardless of the method used for preventing the formation of microemboli, the microembolic signals in the projection of the middle cerebral artery are recorded in all the examined patients, on average 753.4±15.6 IU/surgery or 5,2±0,7 IU/min (for the stage of artificial blood circulation). It should be noted that the formation of microemboli obviously does not have a linear nature. In the vast majority of patients, episodes of group microembolization (from 2-3 to 10 or more signals over a period of 1 to 5 seconds) were observed in connection with surgical manipulations on the aorta, e.g. during cannulation or at the time of overlapping and removal of aortic clamps .

In many cases, group microembolization was observed at the beginning of artificial blood circulation, as well as in restoring effective cardiac activity. Thus, the distribution of the formation of ME was primarily discrete in nature. On the other hand, in some patients, a significant number of microemboli signals were registered throughout the AFC period. They appeared immediately after turning on the machine and disappeared immediately after it stopped, regardless of the events in the operating field. Thus, in many patients, the volume of intraoperative microembolia was determined directly by the functioning of the apparatus of the MS.

It should be noted that changes in photoreaction, signs of a CNS dysfunction and significant hemodynamic abnormalities in any patient were not determined during MI formation.

In assessing the amount of ME in different clinical groups, it has been established that the use of peripheral vasodilators significantly (p<0,05) reduces the risk of microembolisation (Fig. 1)

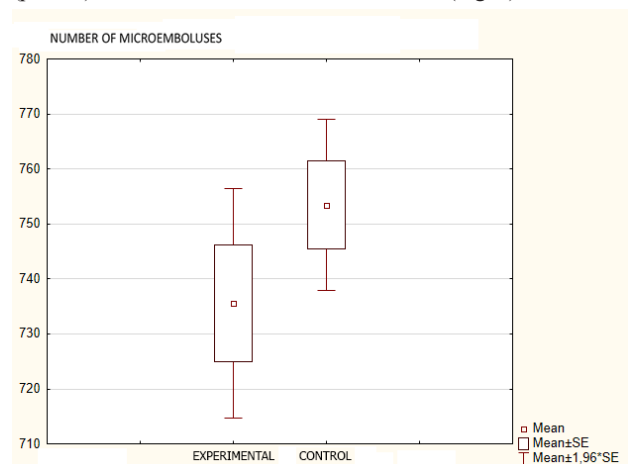


Fig. 1. Development of microemboli during CABG surgery in different clinical groups

As the graph in Fig. 1 shows, there were an average of 753,4±8,0 IU per operation determined, compared to the experimental group with 735,6±10,6 IU. Thus, when using nitroglycerin as a peripheral vasodilator, at a dose of 8-10 µg/kg*min on the background of high (120-130%) volume of perfusion, the number of microemboli in the basin of CNS decreases by 2.4%. However, due to the significant variance of the indicator, the observed differences are not statistically significant (W=0.96, p=0.22). It can be expected that with increasing study group size, the observed trend will be realized in the form of statistically significance.

In the course of the study, a statistically significant strong positive correlation relationship was found between the large number of registered microemboli (>750) in the CNS basin and a decrease in the level of cognition in the early postoperative period (r=0.8 p<0.05) (Fig. 2).

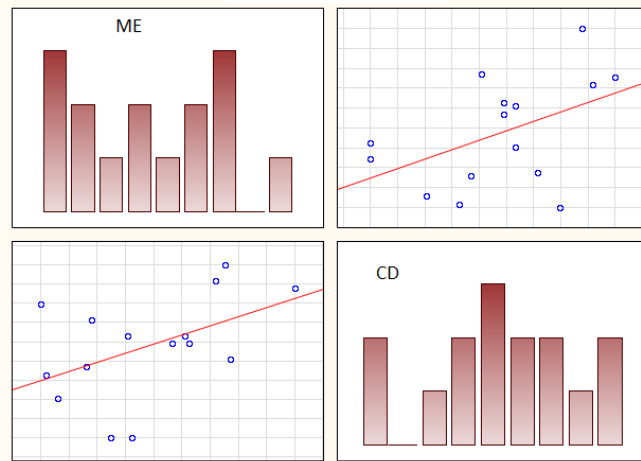


Fig. 2. Relationship between the severity of cognitive dysfunctions and the number of microemboli ($r_s=0.8$)

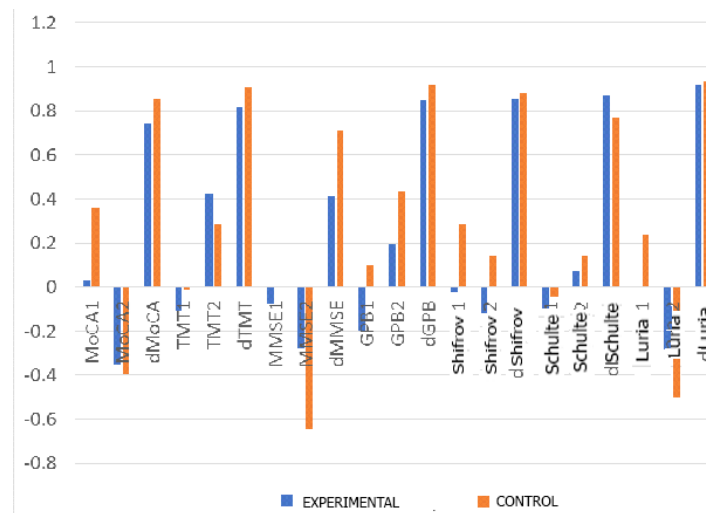


Fig. 3. Correlation relationship between the number of microemboli and cognitive dysfunction indices in patients of the study and control groups

From Fig. 3 it is evident that the most significant correlation was observed with the indicators of reduction of cognitive functions in comparison with the preoperative period (for Δ MoCA - $r_s = 0.74-0.85$, Δ TMT $0.82-0.91$, Δ GPB $0.85-0.92$, Δ of test with coding $0.86-0.88$, Δ of Schulte test $0.77-0.87$, Δ of Luria test $0.92-0.94$). This is consistent with the dynamics of indicators characterizing the cognitive function.

Thus, in the main group Δ MOSA, it was -2.79 ± 0.26 points, Δ d TMT 6.8 ± 0.6 points, Δ dMMSE -2.1 ± 0.2 points, Δ dGPB 22.2 ± 2.1 points, Δ dDifference -3.3 ± 0.2 points, Δ dSound 14.4 ± 0.7 , Δ dLury -10.2 ± 0.7 , which is 15-20% less than in the control group ($p < 0.05$). Thus, the use of vasodilators seems to have a protective effect against the risk of cognitive dysfunction in the early postoperative period.

We deem it noteworthy to point out the view of some authors who consider the causes of intraoperative ischemic damage to the brain not only microembolization within the blood flow, but also systemic hypoperfusion of the brain [21,22]. A number of authors in their studies point to a reliable connection between intraoperative disorders of brain perfusion and deterioration of cognitive status in the postoperative period [23,24].

L. Caplan et al. [25] suggested that the reduction of cerebral perfusion limits the possibility of blood flow from the remov-

al of microemboli from the microcirculatory bed, and that the areas of borderline circulation are particularly susceptible to combined embolic and hypoperfusion injury. Thus, the means of pharmacological correction of cerebral perfusion are of particular importance. In particular, they include nitroglycerin, a pharmaceutical product with a powerful vasodilating effect.

Conclusions. When used as a peripheral vasodilator, nitroglycerin at a dose of $8-10 \mu\text{g}/\text{kg} \cdot \text{min}$ on the background of high (120-130%) volume perfusion rate, the number of microemboli in the basin of CNS decreases by 2.4%, and the severity of the indicators of cognitive dysfunction - by 15-20%

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SUMMARY

PREVENTION OF CEREBROVASCULAR MICROEMBOLIZATION DURING AORTA-CORONARY BYPASS UNDER CONDITIONS OF ARTIFICIAL BLOOD CIRCULATION

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The aim of the research was to investigate the relationship between cerebral microembolization and the development of post-operative cognitive impairment in patients after coronary artery bypass grafting with and without prophylactic administration of nitroglycerin at the end of artificial blood circulation.

The study included 72 patients (43 male and 29 female) who had ACBPS using an ABCA. The patients were randomized into two clinical groups. The number of patients in the control group was 34 patients, in the study group - 38 patients. The study of the cognitive sphere was performed using a battery of clinical tests that included MSA and MMSE scales, Trail-making test, Grooved Pageboard, fine hands-motor test, 10 words test by AR Luria, Wexler's test, Schultz tables. The assessment was carried out on a day before the intervention and on the fifth day after the surgical intervention.

It was shown that by all patients undergoing aorto-coronary bypass surgery microembolization within the cerebral blood flow had been determined. The vast majority of microemboli is formed at the beginning of artificial blood circulation, at the moment of clamping aorta, as well as during the restoration of effective cardiac activity. Intraoperative microembolization of cerebral blood flow in excess of 750 microemboli leads to clinically significant deterioration of the brain functions in the early postoperative period. When used as a peripheral vasodilator, nitroglycerin, at a dose of 8-10 $\mu\text{g}/\text{kg}\cdot\text{min}$ in high (120-130%) volume perfusion rate, decreases the number of microemboluses in the basin of CMA by 2.4%.

Keywords: cerebral microembolization, postoperative cognitive impairment, coronary artery bypass grafting, artificial blood circulation, nitroglycerin.

РЕЗЮМЕ

ПРОФИЛАКТИКА МИКРОЭМБОЛИЗАЦИИ ЦЕРЕБРАЛЬНОГО КРОВотоКА ПРИ ПРОВЕДЕНИИ АОРТОКОРОНАРНОГО ШУНТИРОВАНИЯ В УСЛОВИЯХ ИСКУССТВЕННОГО КРОВООБРАЩЕНИЯ

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Цель исследования - определить связь между церебральной микроэмболизацией и развитием послеоперационных когнитивных расстройств после аортокоронарного шунтирования при назначении нитроглицерина в конце искусственного кровообращения и без его назначения.

Исследованы 72 пациента (43 мужчины и 29 женщин), которым проведено аортокоронарное шунтирование с использованием ИК. Больные распределены в две клинические группы: контрольная (n=34) и основная (n=38). Исследование когнитивной сферы выполняли с помощью клинических тестов: шкалы MoCA и MMSE, Trail-making test, Grooved Pageboard, тест на мелкую моторику рук, заучивание 10 слов по А.Р. Лурия, тест Векслера, таблица Шульце. Оценку проводили за день до и на пятый день после хирургического вмешательства.

Результаты исследования показали, что у всех пациентов, которым было проведено аортокоронарное шунтирование, наблюдалась микроэмболизация церебрального кровотока. Подавляющее большинство микроэмболий образовывалось в начале искусственного кровообращения, в момент

наложения зажима на аорту, а также при восстановлении эффективной сердечной деятельности. Интраоперационная микроэмболизация церебрального кровотока в количестве более 750 микроэмболий приводит к клинически значимому ухудшению функций головного мозга в раннем послеоперационном периоде. При применении нитроглицерина в качестве периферического вазодилатора в дозе 8-10 $\mu\text{g}/\text{kg}\cdot\text{min}$ на фоне высокой (120-130%) объемной скорости перфузии количество микроэмболов в бассейне средней мозговой артерии уменьшается на 2,4%.

რეზიუმე

ცერებრული სისხლის ნაკადის ემბოლიზაციის პროფილაქტიკა აორტოკორონარული შუნტირების დროს ხელოვნური სისხლის მიმოქცევის პირობებში

დ.რადიუშინი, ო.ლოსკუტოვი

პ.შუპიკის სახ. დიპლომის შემდგომი განათლების სამედიცინო აკადემია, ანესთეზიოლოგიისა და ინტენსიური თერაპიის კათედრა, კიევი; ოდესის საოლქო კლინიკური საავადმყოფო, უკრაინა

კვლევის მიზანს წარმოადგენდა კავშირის შეფასება ცერებრულ მიკროემბოლიზაციასა და ოპერაციის შემდგომ კოგნიტიურ დარღვევებს შორის პაციენტებში აორტოკორონარული შუნტირების შემდეგ ხელოვნური სისხლის მიმოქცევის დასასრულს ნიტროგლიცერინის საპროფილაქტიკო შეყვანის გარეშე და პაციენტებში აორტოკორონარული შუნტირების შემდეგ, რომლებსაც პროფილაქტიკის მიზნით ხელოვნური სისხლის მიმოქცევის დასასრულს უკეთებოდა ნიტროგლიცერინი.

კვლევა ჩატარდა 72 პაციენტზე (43 მამაკაცი, 29 ქალი), რომელთაც ჩატარდათ აორტოკორონარული შუნტირება ხს-ს გამოყენებით. პაციენტები რანდომულად განაწილდა ორ კლინიკურ ჯგუფში. საკონტროლო ჯგუფი შეადგინა 34 პაციენტმა, საკვლევის ჯგუფი - 38 პაციენტმა. კოგნიტიური სფეროს გამოკვლევა ჩატარდა კლინიკური ტესტების გამოყენებით, MoCA და MMSE, Trail-making test, Grooved Pageboard შკალების ჩათვლით, ტესტით ხელების წვრილ მოტორიკაზე, ტესტით 10 სიტყვის დასწავლაზე არღურეის მიხედვით, ვეკსლერის ტესტით, შულტეს ტაბულებით. შეფასება ხორციელდებოდა ოპერაციულ ჩარევამდე ერთი დღით ადრე და ჩარევიდან მეხუთე დღე-ღამეს.

ნაჩვენებია, რომ ყველა პაციენტს აღენიშნა ცერებრული სისხლის ნაკადის მიკროემბოლიზაცია. მიკროემბოლების უმეტესობა წარმოიქმნა ხელოვნური სისხლის მიმოქცევის დასასრულს, აორტაზე მომჭერის დადების მომენტში, ასევე, გულის ეფექტური მუშაობის აღდგენისას. ცერებრული ჰემოდინამიკის ინტრაოპერაციული მიკროემბოლიზაცია 750 მიკროემბოლის რაოდენობით იწვევს თავის ტვინის კლინიკურად გამოხატულ დისფუნქციას ადრეულ პოსტოპერაციულ პერიოდში. პერიფერიული ვაზოდილატატორის სახით ნიტროგლიცერინის გამოყენებისას დოზით 8-10 მკგ/კგ/წთ პერფუზიის მაღალი (120 - 130%) მოცულობითი სისქარის ფონზე ტვინის შუა არტერიის ბასეინში მიკროემბოლების რაოდენობა მცირდება 2,4%-ით.

JUVENILE IDIOPATHIC ARTHRITIS AND VITAMIN D STATUS IN UKRAINIAN PATIENTS

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Juvenile idiopathic arthritis (JIA) is not a disease, but an exclusion diagnosis that encompasses all forms of arthritis that begin before the age of 16 years, persist for more than 6 weeks, and are of unknown origin. This heterogeneous group of chronic arthritis has been classified on clinical and laboratory grounds to try to identify homogeneous, mutually exclusives categories suitable for etiopathogenic studies [14].

The etiology of JIA has not been studied enough. Some of risk factors for the disease development are still being investigated. Among them, the role of vitamin D is discussed.

Vitamin D is a secosteroid (pro)-hormone with a wide variety of effects from the classical role as a mediator of calcium and phosphorus metabolism (that plays a significant role in mineralization, growth and remodeling of the bone) [3,9,17] to non- skeleton roles (including modulation of cell growth, neuromuscular and immune function, and reduction of inflammation. Many genes encoding proteins that regulate cell proliferation, differentiation, and apoptosis are modulated in part by Vitamin D) [10,15,17].

As far as Vitamin D receptors (VDRs) in peripheral mononuclear blood cells were discovered, the scientific interest in the role of Vitamin D in modulating immune function has been growing. It was found the causes of Vitamin D in down-regulation of antigen-presenting cells, inhibition of T-cell proliferation, and decreased production of T helper cell-1 cytokines IL-2, interferon gamma and tumor necrosis factor-alpha [5,24]

Plenty of studies demonstrated an association between a Vitamin D deficiency and several autoimmune disorders, such as insulin-dependent diabetes mellitus, systemic lupus erythematosus (SLE), and rheumatoid arthritis (RA) in adult patients [4,11-13]. Single studies have shown that vitamin D plays a significant role in the development of autoimmune diseases also in children [7,18,19].

The VDRs have been found in macrophages, chondrocytes, and synoviocytes in rheumatoid synovium and at sites of cartilage erosion in RA patients [5,12,24].

Vitamin D plays a role in osteoporosis and development of disease activity. However, both of these functions might be common not only in adults with RA but also in children with JIA.

The aim of this study was to assess the possible relationship between the level of vitamin D, main features of patients (age, gender) and clinical characteristics of disease (duration and ac-

tivity of the disease, number of joints involved in pathological process) in JIA patients.

Material and methods. 69 patients with JIA fulfilling current ILAR criteria for the classification of the JIA were examined and 15 healthy children were chosen as the control group. Patients were divided into groups depending on the subtype of the disease. The average age of patients was 10,8 ± 4,6 years. The total duration of the disease was 4 years 1 months ± 1 years 1 month. Selected characteristics of the patients with JIA (Table 1).

The disease activity was analyzed based on juvenile arthritis disease activity score (JADAS27). That score consists of global disease activity assessment by a doctor, global assessment of the child's well-being by the patient/parent, number of joints involved, and ESR value. Patients got methotrexate therapy (15mg/m²). Any of the patients were not treated by corticosteroids. The serum level of 25-hydroxyvitamin D [25(OH)D] was measured through blood test by chemiluminescence method using Cobas 6000, Roche Diagnostics (Switzerland), by taking a blood sample of 5 cc. The use of human biological material was approved by the Ethics Committee of the Institute of children and adolescents, Kharkiv, Ukraine, and written informed consent was obtained according to the Declaration of Helsinki. All statistical analyses were performed using s/n SPSS 17 4a 180844250981. Distributed data was analyzed by linear correlation and regression method. P values less than 0.05 were considered as significant.

Results and their discussion. In this study was examined the vitamin D status in patient with JIA based on general characteristic of patients and features of disease. At the main group the average level of vitamin D in serum was 22,69 ± 7,8 ng/ml, at the control group the vitamin D status was 28,67 ± 5,06 ng/ml. In spite of the fact that a decrease in vitamin D status was observed in both groups, however in the group of healthy children it was significantly higher (p > 0.05). Dependence on gender was not found. The significant difference between level of vitamin D in groups of patients with oligoarticular subtype and polyarticular subtype (p > 0.05) was not found, but status of vitamin D in children with undifferentiated arthritis was lower than in other groups (p < 0,05) (Table 2).

Using the lineal correlation it was not found relationship between vitamin D status and duration of disease (r = 0,12; P > 0,05), activity of disease (r = 0,11; P > 0,05), number of active joints (r = 0,05; P > 0,05) and number of injured joints (r = 0,14; P > 0,05). However, the association between the vitamin D status

Table 1. General characteristic of patients with JIA

Sign	Gender		Total
	male	female	
number of patients	24	45	69
age, years (M ± m)	10,8 ± 4,8	10,9 ± 4,9	10,9 ± 4,8
duration of disease, years (M ± m)	3,0 ± 0,5	4,7 ± 4,8	3,9 ± 2,7
oligoarticular subtype, number of patients, %	9 (6,2)	16 (11,0)	25 (17,3)
polyarticular subtype, number of patients, %	12 (8,3)	22 (15,2)	36 (24,8)
undifferentiated arthritis, number of patients, %	3 (2,1)	7 (4,8)	10 (6,9)

Table 2. Vitamin D status depending on subtype and activity of disease (according to the JADAS27 score) and gender of patients

Sign	Clinical variant of disease								
	oligoarthritic subtype			polyarthritic subtype			undifferentiated arthritis		
gender	male	female	total	male	female	total	male	female	total
duration of disease (M±m)	1,6±2,1	3,3±3,8	3,0±3,4	4,4± 34	6,3±5,5	5,6 ± 4,8	2,9±1,7	2,6± 2,1	2,7 ± 1,9
number of active joints	2,6±1,2	1,9±1,1	2,1±1,2	2,8± 2,1	4,9±2,6	4,7 ± 2,6	1,7±0,6	1,0±0,6	1,2± 0,7
JADAS27	18,3± 5,9	15,7± 6,9	16,6± 6,6	18,8±11,9	23,5± 9,8	21,8±10,7	11,3± 1,2	10,6±6,8	10,8±5,6
level of 25(OH)D, ng/ml	21,7± 5,7	26,4± 7,6	24,7 ±7,2	22,0±9,1	22,0± 7,2	22,3 ± 7,8	18,4± 9,0	19,2±8,6	18,9 ± 8,2

and clinical manifestations of the disease in children has been proved using the regression method. A significant multiple relationship was established between the number of active joints on the one side and the age of patients, duration of disease, the level of vitamin D in serum the number of injured joints, and disease activity on the other side (number of active joints = -1,144+0,005 x age of patients - 0,007x duration of disease + 0,292 x the number of injured joints + 0,033 x level of vitamin D + 0,077 x the number of points accordant to JADAS27).

In our research, we use the cut-off about serum concentrations of 25(OH)D suggested by Grant and Holick [8] as recommended in the revision by the Institute of Medicine [1].

The role of vitamin D in disease activity remains relevant. There is no consensus on this issue at this time. Plenty of recent studies involving adult patients with rheumatoid arthritis demonstrated a considerable reverse relationship between the serum level of vitamin D and severity of disease [19]. What is more, studies reported that children with active disease had lower status of vitamin D than those patients who were in remission or who had less disease activity [7,18].

Other observed researches indicate that vitamin D concentrations are significantly decreased in children with JIA. Decreased vitamin D concentrations may be associated with the pathogenesis of JIA. However, vitamin D concentrations may have no correlations with JIA subtypes, disease severity, and disease activity [6,16,21,2 3,25].

A lot of studies included also a control group consisting of healthy children with matching age and gender. Some of such researches noticed that the percentage of subjects with severe deficiency of vitamin D in the JIA group was significantly higher than that in the control group [23,25]. Others have not found considerable difference between them [20,22].

In this study, the serum level of vitamin D of patients with JIA was lower than normal though. However, we did not find direct correlation between lower 25(OH) D concentrations and severity of disease. Nevertheless, it was proved that disease activity was in regression with age of patients, duration of disease, the number of injured joints and level of vitamin D in children with JIA. It can be noticed that vitamin D status is an important criterion of disease activity as well as age of the patients, duration of disease, the number of injured joints.

Conclusion. The monitoring of vitamin D level is advisable to carry out during observing the children with JIA. It can be useful for timely correction of vitamin D deficiency and preventions both skeleton and non-skeleton complications. That may improve the quality of life of patients and their families.

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SUMMARY

JUVENILE IDIOPATHIC ARTHRITIS AND VITAMIN D STATUS IN UKRAINIAN PATIENTS

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Plenty of studies demonstrated an association between a Vitamin D deficiency and several autoimmune disorders, such as diabetes mellitus, systemic lupus erythematosus (SLE), and rheumatoid arthritis (RA) in adult patients.

This study was aimed to assess probable association between the 25-hydroxyvitamin D [25(OH)D] level and juvenile idiopathic arthritis (JIA) features and the possible relationship between serum vitamin D level and disease activity.

69 patients with JIA were examined and 15 healthy children were chosen as the control group. The mean age of patients was 10 years 8 months \pm 4 years 6 months (45 female, 24 male). 25 patients with oligoarthritic subtype of disease, 34 with polyarthritic subtype and 10 patient with undifferentiated arthritis. The total duration of the disease was 4 years 1 month \pm 1 year 1 month. Patients got methotrexate therapy (15mg/m²).

Any of the patients were not treated by corticosteroids. The serum level of vitamin D was measured through blood test by chemiluminescence method. The relationship between the level of vitamin D and disease activity was analyzed based on juvenile arthritis disease activity score (JADAS27).

The average level of vitamin D in serum was 22,69 \pm 7,8 ng/ml at the control group the vitamin D status was 28,67 \pm 5,06 ng/ml. In spite of the fact that a decrease in vitamin D status was observed in both groups, however in the group of healthy children it was significantly higher ($p > 0.05$). Using the regression method, a significant relationship was established between the number of active joints and the age of patients, duration of disease, the level of vitamin D in serum, the number of injured joints, and disease activity (accordant to JADAS27 score) (number of active joints = - 1,144 + 0,005 x age of patients - 0,007 x duration of disease + 0,292 x the number of injured joints + 0,033 x level of vitamin D + 0,077 x the number of points accordant to JADAS27).

The monitoring of vitamin D level is advisable to carry out during observing the children with JIA. It can be useful for timely correction of vitamin D deficiency and preventions both skeleton and non-skeleton complications. That may improve the quality of life of patients and their families.

Keywords: juvenile idiopathic arthritis, vitamin D status.

РЕЗЮМЕ

ЮВЕНИЛЬНЫЙ ИДИОПАТИЧЕСКИЙ АРТРИТ И СТАТУС ВИТАМИНА Д У УКРАИНСКИХ ПАЦИЕНТОВ

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Целью исследования явилось определение возможной связи уровня витамина Д с особенностями течения ювенильного идиопатического артрита и его влияния на манифестацию заболевания и активность.

Обследованы 69 больных ювенильным идиопатическим артритом (ЮИА), 45 девочек, 24 мальчика, из них 25 пациентов с олигоарткулярным вариантом заболевания, 34 пациента - с полиарткулярным вариантом, 10 - с недифференцированным артритом. Средний возраст пациентов составил 10 лет 8 месяцев \pm 4 года 6 месяцев, средняя длительность заболевания - 4 года 1 месяц \pm 1 год 1 месяц. Все пациенты получали базисную терапию метотрексатом, кортикостероиды у данных больных не применялись. Контрольную группу составили 15 здоровых детей. Уровень витамина Д в сыворотке крови определялся хемилуминесцентным методом. Взаимосвязь между уровнем витамина Д и активностью заболевания анализировалась при помощи шкалы оценки активности ювенильного идиопатического артрита JADAS27.

Средний уровень витамина Д в основной группе составил 22,69 \pm 7,8 нг/мл, в группе контроля - 28,67 \pm 5,06 нг/мл. Несмотря на недостаточность витамина Д в обеих группах, у здоровых детей статус витамина Д был значительно выше ($p > 0.05$). Регрессионным методом выявлена значимая взаимосвязь между количеством активных суставов, возрастом пациентов, длительностью заболевания, уровнем витамина Д в сыворотке

крови, количеством пораженных суставов и степенью активности заболевания согласно шкале JADAS27.

Полученные результаты диктуют необходимость определения уровня витамина D при наблюдении детей с ЮИА для своевременной его коррекции с целью профилактики как скелетных, так и вне скелетных осложнений заболевания и улучшения качества жизни больных детей.

რეზიუმე

იუვენილური იდოპათიური ართრიტი და D ვიტამინის სტატუსი უკრაინაში მცხოვრებ პაციენტებში

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უკრაინის ბავშვთა და მოზარდთა ჯანმრთელობის დაცვის ინსტიტუტი; ხარკოვის ვ.კარაზინის სახელობის ეროვნული უნივერსიტეტი, პედიატრიის №2 კათედრა, უკრაინა

კვლევის მიზანს წარმოადგენდა D ვიტამინის დონის შესაძლო კავშირის განსაზღვრა იუვენილური იდოპათიური ართრიტის მიმდინარეობის, დაავადების მანიფესტირების და აქტივობის თავისებურებებთან,

გამოკვლეულია 69 ავადმყოფი იუვენილური ართრიტი (45 გოგონა, 24 ვაჟი), მათგან 25 პაციენტი – დაავადების ოლიგოარტიკულური ვარიანტით, 34 – პოლიარტიკულური ვარიანტით, 10 – არადიფერენ-

ცირებული ართრიტით. პაციენტების საშუალო ასაკი იყო 10 წელი,8 თვე \pm 10 წელი,6 თვე, დაავადების საშუალო ხანგრძლივობა - 4 წელი,1 თვე \pm 1 წელი,1 თვე. ყველა პაციენტი იღებდა ბაზისურ თერაპიას მეტოტრექსატით; კორტიკოსტეროიდები ამ პაციენტებთან არ გამოიყენებოდა. საკონტროლო ჯგუფი შეადგინა 15 ჯანმრთელმა ბავშვმა. D ვიტამინის დონე სისხლის შრატში განისაზღვრა ქემილუმინესცენტური მეთოდით. კავშირი D ვიტამინის დონესა და დაავადების აქტივობას შორის გაანალიზებულია იუვენილური იდოპათიური ართრიტის აქტივობის შეფასების შკალით JADAS27.

D ვიტამინის საშუალო დონემ ძირითად ჯგუფში შეადგინა 22,69 \pm 7,8 ნგ/მლ, საკონტროლო ჯგუფში - 28,67 \pm 5,06 ნგ/მლ. მიუხედავად D ვიტამინის უკმარისობისა ორივე ჯგუფში, ჯანმრთელ ბავშვებთან D ვიტამინის სტატუსი მნიშვნელოვნად მაღალი იყო ($p>0.05$). რეგრესიული მეთოდით გამოვლენილია კავშირი აქტიური სახსრების რაოდენობას, პაციენტის ასაკს, დაავადების ხანგრძლივობას, სისხლის შრატში D ვიტამინის დონეს, დაზიანებული სახსრების რაოდენობას და დაავადების აქტივობას შორის JADAS27- შკალის მიხედვით.

მიღებული შედეგები მიუთითებს D ვიტამინის დონის განსაზღვრის აუცილებლობაზე იუვენილური იდოპათიური ართრიტის მქონე ბავშვებში ჩონჩხოვანი და არაჩონჩხოვანი გართულებების დროული კორექციისა და სიცოცხლის ხარისხის გაუმჯობესებისათვის.

ФЕНОТИПИЧЕСКИЙ ПОЛИМОРФИЗМ N-АЦЕТИЛТРАНСФЕРАЗЫ 2 У БОЛЬНЫХ САХАРНЫМ ДИАБЕТОМ

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Ариламин N-ацетилтрансферазы являются уникальной группой ферментов, которая играет значимую роль в детоксикации лекарственных средств и активации канцерогенов. У человека присутствуют два изофермента - NAT1 и NAT2, кодируемые полиморфными генами. Значительные вариации ацетилирования лекарств связаны с полиморфизмом гена NAT2 [1], который генерирует быстрые, промежуточные и медленные фенотипы ацетилирования через изменения структуры фермента; поэтому, человеческую популяцию на основе их ацетиляционных возможностей можно разделить на подгруппы [4].

С одной стороны, генотип медленного ацетилятора (АЦ) может являться значимой детерминантой развития и клинического течения пародонтита, сахарного диабета, бронхиальной астмы, рака мочевого пузыря [1,5,9]. Среди других соматических патологий установлены ассоциации между NAT2 и кардиоваскулярной патологией в китайской популяции: генотип медленного АЦ является фактором риска ишемической болезни сердца [9].

Результаты исследования Irshaid YM, Abujbara MA [8] показали, что генотипы медленных АЦ NAT2 могут яв-

ляться значимой генетической детерминантой в развитии СД у турецкого населения. При этом в популяции существует избыток генотипов, которые кодируют промежуточное ацетилирование у больных СД 2 типа и избыток генотипов с медленным ацетилированием у пациентов с СД 1 типа [7]. С другой стороны, NAT2 полиморфизм имеет значение для скорости инактивации и, соответственно, концентрации различных лекарственных препаратов, включая изониазид, дапсон, прокаинамид и сульфаметазин [1,4,5]. Таким образом, определение генотипа/фенотипа NAT2 может служить вспомогательным методом как для повышения эффективности фармакотерапии и минимизации побочных эффектов при лечении ряда заболеваний [1], так и для определения рисков возникновения различных патологий. Значительные отличия в полиморфизме NAT2 наблюдаются среди различных этнических групп, однако среди жителей Украины имеются весьма скудные данные об этом полиморфизме. Определение генотипов ацетилирования, как рутинного исследования в условиях лечебных учреждений, по сей день остается невозможным. Поиск новых методов определе-

ния фенотипа ацетилирования, не требующих больших финансовых затрат и активного вмешательства, является перспективным направлением исследований.

Цель исследования определить распределение генетически детерминированных признаков в зависимости от ацетиляторного фенотипа у больных сахарным диабетом в сравнении со здоровыми лицами и разработать методику определения фенотипа ацетилирования без биохимического вмешательства.

Материал и методы. Открытое проспективное рандомизированное клиническое исследование проведено на базе Винницкого областного клинического высокоспециализированного эндокринологического центра. Информированное согласие получено у всех пациентов. Критериями исключения из исследования являлись острые и осложненные формы ИБС и гипертонической болезни (стенокардия, возникшая впервые, острый инфаркт миокарда, острое нарушение мозгового кровообращения, недостаточность кровообращения IIБ-III ст., блокады сердца и нарушение сердечного ритма); больные СД 2 типа, которые до начала исследования получали инсулинотерапию, диабетические осложнения (нефропатия IV-V ст., ретинопатия III ст., выраженные ангио- и нейропатия конечностей), хронические заболевания легких, почек и желудочно-кишечного тракта в фазе обострения и с тяжелым течением, возраст более 70 лет, хронический алкоголизм, отказ больного принимать участие в исследовании.

В исследование включено 115 больных СД 2 типа в возрасте $57,49 \pm 1,23$ г. Контрольную группу составили практически здоровые лица ($n=141$) с нормальным уровнем глюкозы в крови, среди них 73 (51,8%) женщины и 68 (48,2%) мужчин, в возрасте от 20 до 32 лет (средний возраст $38,3 \pm 1,77$ года).

Установление типа ацетилирования проводили путем определения метаболитов сульфадимезина по методу Брайтона и Маршала. Кроме того, среди больных с определенным ацетиляторным статусом проводили измерение антропометрических и антропоскопических показателей (обхватные и диаметральные размеры конечностей, грудной клетки, шеи и головы, ширина жировых складок на тех же частях тела, форма ногтей, цвет глаз и волос), генетически детерминированных признаков (группа крови, резус-фактор, наличие ямочек на подбородке и щеках, умение скручивать язык в трубочку, наличие или отсутствие приращенной мочки уха).

Математическую обработку полученных данных проводили с помощью стандартных методов вариационного анализа с применением пакета программ Statistica 6.0. Результаты представлены в виде «среднее значение (M)

\pm погрешность (m)». Для оценки межгрупповой разницы применяли параметрический t-критерий Стьюдента и χ^2 для непараметрических показателей. За достоверность различий в группах принят уровень статистической значимости $p < 0,05$. Регрессионный анализ проведен с применением статистического пакета StatSoft «Statistica», версия 5.5 (лицензионный № SN AXXR910A374605FA).

Результаты и их обсуждение. Определение активности NAT 2 среди 115 больных СД выявило бимодальное распределение фенотипического полиморфизма фермента в соотношении быстрых к медленным АЦ как 45,2% ($n=52$) к 54,8% ($n=63$).

Одинаковым было соотношение ацетиляторного полиморфизма среди обоих полов. Среди мужчин выявлено 27 (57,4%) медленных и 20 (42,6%) быстрых АЦ и, соответственно, среди женщин - 36 (52,9%) медленных и 32 (47,1%) быстрых. Разница в распределении ацетиляторного статуса по полу недостоверна ($t < 2,0$; $p > 0,05$).

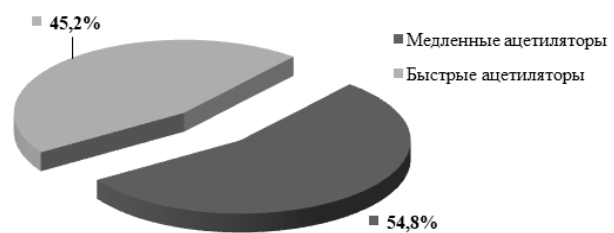


Рис. 1 Структура полиморфизма NAT2 у больных СД 2 типа (в %)

Подобная картина наблюдалась среди 141 здорового представителя популяции Подольского региона: соотношение медленных к быстрым АЦ как 79 (56%) к 62 (44%), что повторялось среди обоих полов.

Проведен анализ взаимосвязи ацетиляторного статуса с генетически детерминированными признаками, постоянными для человеческого организма: группа крови, резус-фактор, наличие ямочек на подбородке и щеках, умение скручивать язык в трубочку, наличие или отсутствие приращенной мочки уха, форма ногтей, цвет волос и глаз.

Анализ взаимосвязи ацетиляторного статуса со структурой групп крови и резус-фактора достоверной разницы среди того или иного типа ацетилирования не выявил. У больных СД 2 типа, как и в общей популяции, соотношение I и II групп крови было 34,7%/41,7%, а III и IV групп - 15,3% и 8,3%, соответственно. При оценке распределения резус-фактора обнаружено 81,9% резус-положительных лиц и 18,1% резус-отрицательных. Такой же была структура

Таблица 1. Распределение групп крови и резус-фактора в зависимости от полиморфизма NAT2 у больных СД 2 типа

Группа крови, резус фактор (Rh)	Ацетиляторный статус (n=115)		В общей выборке больных СД
	Медленные АЦ (n=63)	Быстрые АЦ (n=52)	
I группа (0)	33,3%	36,4%	34,7%
II группа (A)	46,2%	36,4%	41,7%
III группа (B)	15,4%	15,2%	15,3%
IV группа (AB)	5,1%	12,0%	8,3%
Rh+	82,1	81,8	81,9%
Rh-	17,9	18,2	18,1%

групп крови и резус-фактора среди обоих ацетиляторных фенотипов, без достоверной разницы ($t < 1,0$; $p > 0,05$).

Обнаружена достоверная разница в структуре таких генетических признаков, как наличие ямочек на подбородке и щеках, умение скручивать язык в трубочку и наличие или отсутствие приращенной мочки уха, между здоровыми лицами и больными СД (таблица 2).

Это же соотношение наблюдается и при различных типах ацетилирования у здоровых и больных СД. Разница в структуре данных признаков между медленными и быстрыми АЦ недостоверна ($t < 2$; $p > 0,05$).

Анализ связи формы ногтей с ацетиляторным статусом не показал достоверной разницы в соотношении данного показателя среди медленных и быстрых АЦ.

Среди больных СД 2 типа выявлено равное соотношение лиц с разным цветом волос. У пациентов с СД достоверно увеличена доля лиц с черными волосами (брюнетов) в сравнении с практически здоровыми, причем эта закономер-

ность сохраняется среди быстрых и медленных АЦ (таблица 3).

Анализ распределения пациентов с СД по цвету глаз обнаружил достоверную разницу в структуре данного показателя среди медленных и быстрых АЦ: среди быстрых АЦ увеличена доля больных с карими глазами и уменьшена - с голубыми, против пациентов с медленным ацетиляторным статусом (таблица 4).

Среди обследованного контингента больных подавляющее большинство - 87 (75,7%), имели ту или иную степень ожирения и только 24,3% пациентов с СД имели нормальную массу тела. Достоверной разницы в распределении массы тела среди медленных и быстрых АЦ не обнаружено ($t < 2$; $p > 0,05$).

При анализе связи наследственных факторов и фенотипов ацетилирования достоверной разницы в распределении семейных заболеваний среди быстрых и медленных АЦ больных СД не обнаружено ($t < 2,0$; $p > 0,05$), таблица 5.

Таблица 2. Взаимосвязь между ацетиляторным статусом и генетическими детерминантами у больных СД 2 типа

Признак	Полиморфизм NAT2 (n=115)		Всего среди больных СД
	Медленные АЦ (n=63)	Быстрые АЦ (n=52)	
Ямочки на подбородке: есть	54,0%	46,2%	50,4%**
нет	46,0%	53,8%	49,6%**
Ямочки на щеках: есть	55,6%	53,8%	54,8%**
нет	44,4%	46,2%	45,2%**
Умение скручивать язык в трубочку: есть	39,7%	28,8%	34,8%**
нет	60,3%	71,2%	65,2%**
Мочка уха: приращенная	49,2%	61,5%	54,8%*
не приращенная	50,8%	38,5%	45,2%*

примечания: * - достоверная разница в распределении соответствующих признаков между практически здоровыми лицами и больными СД ($t > 3,0$; $p < 0,01$);
** - достоверная разница в распределении соответствующих признаков между практически здоровыми лицами и больными СД ($t > 5,0$; $p < 0,001$)

Таблица 3. Ассоциации ацетиляторного статуса и цвета волос среди больных СД 2 типа

Цвет волос	Ацетиляторный статус (n=115)		Всего среди больных СД
	Медленные АЦ (n=63)	Быстрые АЦ (n=52)	
Русый	34,2%	40,5%	37,5%
Шатен	36,9%	32,4%	35,0%
Брюнет	28,9%	26,2%	27,5%*

примечание: * - достоверная разница соотношения по цвету волос между здоровыми лицами и больными СД ($t > 2,0$; $p < 0,05$)

Таблица 4. Распределение цвета глаз среди различных типов ацетилирования у больных СД

Цвет глаз	Полиморфизм NAT2 (n=115)		Всего
	Медленные АЦ (n=63)	Быстрые АЦ (n=52)	
голубой	19,4%	2,6%*	10,1%
серый	32,2%	31,6%	31,9%
зеленый	22,6%	13,2%	17,4%
карий	25,8%	52,6%*	40,6%

примечание. * - достоверная разница соотношения цвета глаз между медленными и быстрыми АЦ ($t > 2,0$; $p < 0,05$)

Таблица 5. Ассоциация фенотипов метаболизма у больных СД 2 типа с частотой различных заболеваний в их семьях

Заболевания в семьях	Больные СД 2 типа (n=115)				Всего среди больных СД	
	Медленные АЦ		Быстрые АЦ		абс.	%
	абс.	%	абс.	%		
Сердечно-сосудистые	53	84,1	47	90,4	100	87
Аллергические и бронхолегочные	16	25,4	13	25	29	25,2
Нарушения обмена	27	42,9	24	46,2	51	44,3
Патология сосудов и соединительной ткани	6	9,5	3	5,8	9	7,8
Заболевания желудочно-кишечного тракта	10	15,9	8	15,4	18	16,7
Доброкачественные и злокачественные новообразования	13	20,6	19	36,5	32	27,8

Литературные данные о распределении фенотипов ацетилования среди больных СД противоречивы, они обусловлены неоднородностью популяций. Полиморфный характер метаболизма сульфадимезина среди больных СД в пределах Украины в данном исследовании показан впервые. Существенных различий в фенотипическом составе среди обследованных групп не выявлено, что свидетельствует о жестком генетическом контроле NAT2 и отсутствии ассоциаций между ацетиляторным статусом и заболеванием в качестве предиктора, а следовательно, необходимы поиски его связей с другими генетическими маркерами.

Достоверных корреляций между генетическими маркерами, антропометрическими показателями, как отдельными единицами, и ацетиляторным полиморфизмом не обнаружено. Поэтому дальнейший поиск предполагает определить наличие ассоциативных связей одно- и многофакторного направления, что позволит обнаружить конкретные параметры для определения фенотипа ацетилования по внешним генетически детерминированным признакам.

Для разработки прогностической модели и шкалы прогнозирования характера ацетилования задействована статистическая матрица, которая объединила результаты обследования трех групп: I - здоровые лица (n=141) II - пациенты с ИБС (n=85) и III - больные с СД 2 типа в сочетании с ИБС (n=72) и включала 46 клинико-антропологических показателей, перечисленных выше. Все 3 группы показали бимодальное распределение фенотипического полиморфизма фермента в соотношении быстрых к медленным АЦ без достоверной межгрупповой разницы.

При помощи пошаговой многофакторной регрессии («Stepwise Variable Selection») получено 12 моделей линейной многофакторной регрессии типа: $Y=f(A, B, \dots, L)$, где в качестве независимых переменных A, B, ...L выступили параметры, которые характеризуют антропологический и клинический статус пациентов, а в качестве выходного параметра (Y) - характер ацетилования (показатель закодирован в виде баллов, где 1 балл - медленные АЦ и 2 балла - быстрые АЦ) [10].

Полученная модель предоставляет возможность прогнозировать характер ацетилования с помощью 11 антропологических факторов и группы крови пациента. Так, на характер ацетилования (величина выходного параметра Y) в подавляющем большинстве влияли антропологические факторы: цвет глаз и волос, форма мочки

уха и подбородка, размер дистального эпифиза голени, обхватные размеры голени, грудной клетки и плеча, а также толщина жировой складки на животе, под лопаткой и на предплечье. Из клинических данных единственным параметром, который влиял на характер ацетилования, оказалась группа крови. Приведенные данные выявили генетически обусловленную направленность характера ацетилования в проанализированных выборках пациентов.

С целью упрощения методики определения характера ацетилования путем сокращения количества различных показателей разработана балльная шкала прогнозирования ацетиляторного статуса с помощью независимых антропологических факторов. Поэтому проведен анализ силы влияния отдельных регрессоров (независимых переменных модели) на выходной параметр Y и значимости этих воздействий, что характеризовалось величиной β -коэффициента и p. Только 6 переменных проявляли статистически значимое ($p < 0,041$) влияние на выходной параметр. Другие переменные не показали значимого влияния на выходной параметр ($p > 0,058$) и исключены нами из дальнейшего анализа (таблица 6). Пропорционально β -коэффициентам конечной модели были назначены соответствующие баллы. При этом переменной с наименьшим β -коэффициентом (в нашем случае наименьший β -коэффициент = 0,08) был назначен 1 балл, остальные баллы определяли путем деления соответствующих β -коэффициентов на наименьший β -коэффициент с последующим округлением доли до целого числа.

Следует отметить, что самый большой балл (5 баллов) в разработанной шкале имел показатель G, который характеризовал толщину жировой складки на животе. В свою очередь, наименьший балл (2 балла) имели два показателя: C - размер дистального эпифиза голени и I - цвет волос (брюнет либо не брюнет).

Таким образом, в разработанную шкалу прогнозирования характера ацетилования включены 6 независимых факторов. Минимальное количество баллов, которое могло определяться согласно шкалы, составило 0 и максимальное - 19 баллов.

С помощью однофакторного анализа и уравнений линейной регрессии, которые показывали характер зависимости различных переменных от исходного параметра, рассчитаны критические величины для независимых факторов. Расчеты проведены отдельно для больных с медленным и быстрым типом ацетилования (таблица 7).

Таблица 6. Независимые переменные, влияющие на характер ацетилирования, и анализ вклада отдельных факторов в эффективность прогнозирования

Переменные	Параметры	β -коэффициент	P	Баллы
A	Цвет глаз (1 – карий, 0 – не карий)	-0,11	0,067	-
B	Форма мочки уха (1 - фиксированная мочка уха, 0 – не фиксированная)	0,11	0,058	-
C	Размер дистального эпифиза голени (см)	0,18	0,007	2
D	Наличие ямочки на подбородке (1 – есть ямочка, 0 – нет ямочки)	- 0,11	0,070	-
E	Группа крови	0,08	0,14	-
F	Обхватный размер голени (см)	-0,12	0,073	-
G	Толщина жировой складки на животе (см)	0,39	0,001	5
H	Толщина жировой складки под лопаткой (см)	-0,27	0,011	3
I	Цвет волос (1 – брюнет, 0 – не брюнет)	- 0,12	0,041	2
J	Толщина жировой складки на предплечье (см)	- 0,10	0,084	-
K	Обхватный размер грудной клетки на выдохе (см)	0,29	0,014	4
L	Обхватный размер плеча (см)	-0,21	0,027	3

Таблица 7. Критические величины независимых переменных при разных вариантах ацетилирования

Независимые факторы	Баллы	Критические величины	
		Медленные АЦ	Быстрые АЦ
Размер дистального эпифиза голени (см)	2	<6,5	>6,5
Толщина жировой складки на животе (см)	5	<2,1	>2,0
Толщина жировой складки под лопаткой (см)	3	>2,0	<1,7
Цвет волос	2	Брюнет	Не брюнет
Обхватные размеры грудной клетки на выдохе (см)	4	99	> 02
Обхватные размеры плеча (см)	3	>33	<33

Таблица 8. Эффективность прогнозирования характера ацетилирования по прогностической шкале

Баллы	Прогнозирование медленных АЦ			Прогнозирование быстрых АЦ		
	Инф.	Чувст.	Спец.	Инф.	Чувст.	Спец.
2 и >	52%	52%	100%	48%	48%	0%
3 и >	53%	53%	62%	47%	47%	29%
5 и >	53%	53%	58%	48%	48%	47%
6 и >	56%	55%	62%	48%	48%	50%
7 и >	59%	58%	62%	46%	47%	44%
8 и >	58%	58%	59%	47%	47%	48%
9 и >	53%	55%	51%	54%	52%	56%
10 и >	53%	57%	51%	55%	53%	56%
11 и >	53%	58%	51%	55%	54%	55%
12 и >	46%	37%	47%	57%	65%	56%
13 и >	47%	40%	47%	57%	67%	56%
14 и >	47%	29%	47%	54%	80%	53%
15 и >	47%	25%	48%	53%	100%	53%

Ниже приводится пример пациентки П.: женщина, 23 года, размер дистального эпифиза голени равен 7 см - 2 балла; толщина жировой складки на животе - 1,6 см - 5 баллов; толщина жировой складки под лопаткой - 1,2 см - 3 балла; цвет волос - русые - 2 балла; обхватный размер грудной клетки на выдохе - 108 см - 4 балла; обхватный размер плеча - 30 см - 3

балла. Сумма данных показателей составила 15 баллов, следовательно со 100% вероятностью можно прогнозировать быстрый тип ацетилирования. По данным сульфадимезинового теста содержание N-ацетилсульфадимезина в моче составил: 0-6 ч. - 73%, 7-12 ч. - 93%. Итак, как по антропологическим показателям, математически обработанными

согласно бальной шкалы прогнозирования, так и по результатам биохимического тестирования данное лицо является быстрым АЦ.

Согласно разработанной шкалы, медленный тип ацетилирования следовало предвидеть у больших-брюнетов с размером дистального эпифиза голени менее 6,5 см, толщиной жировой складки на животе менее 2,1 см и толщиной жировой складки под лопаткой более 2,0 см, обхватным размером грудной клетки на выдохе менее 99 см и обхватным размером плеча более 33 см. Быстрый тип ацетилирования следовало ожидать у больших небрюнетов, с размером дистального эпифиза голени более 6,5 см, толщиной жировой складки на животе более 2 см и толщиной жировой складки под лопаткой менее 1,7 см, при обхватных размерах грудной клетки на выдохе более 102 см и обхватных размерах плеча менее 33 см.

В таблице 8 приведены результаты анализа эффективности прогнозирования характера ацетилирования по разработанной прогностической шкале (критерии приведены в таблице 7) отдельно для медленных и быстрых АЦ

При прогнозировании медленного типа ацетилирования и наличии 2 баллов имеет место 100% специфичность прогноза. Последнее свидетельствует, что при наличии 2 баллов со 100% вероятностью возможно исключение наличия у больных быстрого типа ацетилирования. Рост количества баллов (3 и более) приводит к существенному уменьшению специфичности прогнозирования медленного типа ацетилирования. Наибольшая чувствительность (55-58%) прогнозирования медленного типа ацетилирования определялась в пределах от 6 до 11 баллов.

С другой стороны, данные таблицы 8 свидетельствуют о достаточно высокой (65% и выше) чувствительности прогнозирования быстрого типа ацетилирования при наличии 12 и более баллов по разработанной шкале. При наличии 15 баллов чувствительность прогнозирования составила 100%.

Заключение. В результате проведенного исследования выявлено бимодальное распределение фенотипического полиморфизма активности N-ацетилтрансферазы 2 как среди больных сахарным диабетом, так и здоровых лиц без гендерных различий.

Генетический анализ больных СД 2 типа и здоровых лиц молодого возраста выявил следующие особенности:

- у здоровых лиц медленный фенотип NAT2 достоверно чаще встречается у лиц с коричневым цветом глаз; быстрый фенотип NAT2 - достоверно чаще у сероглазых; быстрые АЦ вероятно имели в семейном анамнезе нарушения обмена веществ (сахарный диабет, ожирение);
- среди лиц с быстрым АЦ и карими глазами в 2 раза чаще встречается СД 2 типа, а с голубыми глазами - в 7 раз реже, чем у пациентов с медленным ацетилирующим статусом.

Вышеизложенное подчеркивает наличие связи между ацетилирующим статусом и антропологическими данными. Неоднородность в распределении генетических детерминант среди ацетилирующих фенотипов, по всей вероятности, обусловлена разницей в соотношении данных признаков среди здоровых лиц и больных.

Среди больных СД достоверно увеличена доля наличия ямочек на подбородке и щеках, приращенной мочки уха в сравнении с контрольной группой; уменьшен удельный вес пациентов с умением скручивать язык в трубочку и в 1,8 раза увеличен - с черным цветом волос. Вышеприведенные данные подчеркивают наличие генетического перекрестного характера наследования

приведенных заболеваний в ассоциации с антропологическими факторами.

Нами впервые разработана шкала прогнозирования фенотипа ацетилирования, которая позволяет определять ацетилирующий статус с помощью антропометрических показателей, без биохимического вмешательства.

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SUMMARY

PHENOTYPIC POLYMORPHISM OF N-ACETYLTRANSFERASE 2 IN PATIENTS WITH DIABETES MELLITUS

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Polymorphism of N-acetyltransferase 2 (NAT2) determines the risk of certain diseases and the rate of inactivation of various xenobiotics and drugs. We investigated phenotypic polymorphism of N-acetyltransferase 2 among 141 healthy subjects and

115 patients with type 2 diabetes mellitus. An analysis of the distribution of genetic determinants, depending on the acetylation status, was carried out. The bimodal distribution of the phenotypic polymorphism of NAT 2 activity was revealed among diabetic patients and healthy individuals. There was no gender difference in NAT2 activity in both groups of subjects. Genetic analysis showed a prevalence of brown-eyed diabetic patients compared to blue-eyed persons among fast acetylators. Among diabetic patients prevailed subjects with dimples on the chin and cheeks and the enlarged ear lobe as well as the reduced proportion of patients with the ability to twist the tongue into a tube compared to healthy individuals. There was a significantly increased proportion of black-haired persons among diabetic patients compared to healthy individuals. Acetylation phenotype prediction scale developed was developed, which allows determining the acetylator status without biochemical intervention, but using anthropometric indicators.

Keywords: N-acetyltransferase 2, genetic determinants, diabetes mellitus type 2.

РЕЗЮМЕ

ФЕНОТИПИЧЕСКИЙ ПОЛИМОРФИЗМ N-АЦЕТИЛТРАНСФЕРАЗЫ 2 У БОЛЬНЫХ САХАРНЫМ ДИАБЕТОМ

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Полиморфизм N-ацетилтрансферазы 2 (NAT2) определяет риск возникновения некоторых заболеваний и скорость инактивации различных ксенобиотиков и лекарственных препаратов. Исследован фенотипический полиморфизм NAT2 у здоровых лиц (n=141) и больных сахарным диабетом (СД) 2 типа (n=115). Проведен анализ распределения генетических детерминант в зависимости от ацетиляторного статуса. Выявлено бимодальное распределение фенотипического полиморфизма активности NAT2 как среди больных СД, так и здоровых лиц без гендерных различий. Особенности генетического анализа при СД показали достоверное увеличение доли больных СД с карими глазами и уменьшение с голубыми среди быстрых ацетиляторов. Среди больных СД достоверно увеличена доля наличия ямочек на подбородке и щеках и приращенной мочки уха в сравнении со здоровыми лицами, уменьшен удельный вес пациентов с умением скручивать язык в трубочку; в сравне-

нии со здоровыми у пациентов с СД достоверно увеличен удельный вес больных с черным цветом волос. Разработана шкала прогнозирования фенотипа ацетилирования, которая позволяет определять ацетиляторный статус с помощью антропометрических показателей, без биохимического вмешательства.

რეზიუმე

N-აცეტილტრანსფერაზა 2-ის ფენოტიპური პოლიმორფიზმი შაქრიანი დიაბეტით დაავადებულებში

ი.დოროშკევიჩი, ო.იაკოვლევა, ო.კირიჩენკო, ა.ჯამბა, ე.პივტორაკი

ვინიცას ნ.პიროგოვის სახელობის ეროვნული სამედიცინო უნივერსიტეტი, უკრაინა

N-აცეტილტრანსფერაზა 2-ის (NAT2) პოლიმორფიზმი განსაზღვრავს ზოგიერთი დაავადების განვითარების რისკს და სხვადასხვა ქსენობიოტიკის და სამკურნალო პრეპარატების ინაქტივაციის სიჩქარეს.

NAT2-ის ფენოტიპური პოლიმორფიზმი გამოკვლეულია ჯანმრთელ (n=141) და შაქრიანი დიაბეტი ტიპი 2-ით დაავადებულ (n=115) პირებში. გენეტიკური დეტერმინანტების განაწილების ანალიზი ჩატარებულია აცეტილტრანსფერული სტატუსის მიხედვით. გამოკვლენილია NAT2-ის ფენოტიპური პოლიმორფიზმის ბიომოლეკულური განაწილება როგორც შაქრიანი დიაბეტით დაავადებულებში, ასევე, ჯანმრთელ პირებში, გენდერული განსხვავებების გარეშე. გენეტიკური ანალიზის თავისებურებანი შაქრიანი დიაბეტის დროს მიუთითებს სწრაფ აცეტილარტა შორის ამ დაავადების მქონე ყავისფერი თვალის ფერის ადამიანების წილის გაზრდაზე და ცისფერთვალბათა წილის შემცირებაზე. შაქრიანი დიაბეტით დაავადებულთა შორის, ჯანმრთელ ადამიანებთან შედარებით, გაზრდილია ნიკაპსა და ლოყებზე მცირე ზომის ღრმულის და ყურის შეზრდილი ბიბილოს მქონეთა წილი, შემცირებულია პაციენტების რაოდენობა, ვისაც შეუძლია ენის მიღისებურად დახვევა; ჯანმრთელ ადამიანებთან შედარებით, შაქრიანი დიაბეტით დაავადებულთა შორის გაზრდილია შავთმიან პირთა ხვედრითი წილი.

შემუშავებულია აცეტილირების ფენოტიპის პროგნოზირების შკალა, რომელიც იძლევა აცეტილტრანსფერული სტატუსის განსაზღვრის საშუალებას ანთროპომეტრიული მაჩვენებლების მიხედვით, ბიოქიმიური კვლევის გარეშე.

ПРОГНОСТИЧЕСКОЕ ЗНАЧЕНИЕ БИОМАРКЕРОВ КРОВИ И БРОНХОАЛЬВЕОЛЯРНОГО ЛАВАЖА ДЛЯ ИДИОПАТИЧЕСКОГО ЛЕГОЧНОГО ФИБРОЗА (ОБЗОР)

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Идиопатический легочный фиброз (ИЛФ), известный ранее в пульмонологии как идиопатический фиброзирующий альвеолит – синдром Хаммана-Рича (описанный в 1935, 1944 году Hamman L., Rich A.R.) остается лидером среди заболеваний с неизвестной этиологией, диагностика и лечение которых малоуспешны, несмотря на прогресс молекулярной медицины. Скоротечное неблагоприятное завершение ИЛФ позволяет его считать даже более смертельным заболеванием, чем многие виды рака. Неутешительный прогноз и высокая смертность в течение 2-5 лет после диагностики [15,16] ставят перед необходимостью поиска эффективных методов терапии [12], среди которых наиболее успешной является трансплантация легких [29, 39]. Однако длительные списки ожидания и последующий результат могут быть «перечеркнуты» скрытыми в организме особенностями молекулярных регуляторных механизмов, нивелирующих достигнутый положительный эффект пересадки. К другим методам поддерживающей терапии можно отнести длительную кислородотерапию, реабилитационные мероприятия и недостаточно успешную фармакологическую поддержку.

Предшествующие разновекторные аспекты изучения патогенеза ИФЛ по сей день не выявили ключевые механизмы нарушений. Длительное скрытое течение до верификации диагноза, иногда возможное только при биопсии легких, позволяет предположить, что какое-либо первичное повреждение легочной ткани, эпителия или эндотелия инициирует каскады профиброгенных реакций через активацию клеток воспаления и формирование фиброза, т.к. биологическое значение рубцовой ткани направлено на ограничение агрессивного патологического процесса. Поэтому диагностика ИФЛ реализуется уже на конечных этапах лишь следов воспаления, чем и определяется неэффективность глюкокортикоидов (ГК) или цитостатиков.

Роль воспалительного и инфекционного начала. Среди патогенетических аспектов развития ИФЛ ранее предполагалась роль инфекционного фактора, однако морфологические исследования и характер клеточных реакций, отсутствие эффективности противовоспалительной терапии не подтвердили такую возможность, тем не менее, значение инфекционного начала нельзя исключить полностью. Так, наблюдения свидетельствуют, что у пациентов с ИФЛ довольно часто встречается продромальный вирусный синдром ещё до развития респираторных симптомов [14]. Предполагаемыми вирусами могут быть и вирусы гепатита С, однако выводы достаточно противоречивы в отношении его триггерной роли при ИЛФ. Гораздо больше внимания уделено вирусам семейства герпеса человека (HHV). В работах Tang Y.W. et al. [38] показано, что в 97% случаев ИЛФ наблюдалась предшествующая инфекция, вызванная одним или двумя видами вируса этого семейства (против контроля всего 36%). Возможно присутствие вирусов и в самой легочной ткани больных: в альвеолярном эпителии выявлены латентные литические вирусы HHV, тем более, что гипотеза успешно подтверждена экспериментально, особенно у стареющих мышей [28]. Среди механизмов такого влияния

называют вирусный стресс эндоплазматического ретикула легочного эпителия, экспрессию фактора роста опухоли TGF-b (мощного профиброгенного фактора) или протеинов сурфактанта [28,36]. Однако в наблюдениях авторов [43] присутствие вирусов в тканях легких отрицается даже в случаях смерти [24].

Хотя бактериальные инфекции респираторных путей при госпитализации таких пациентов несут риск смерти, роль бактериальной флоры изучена недостаточно, тем более что существует давнее ложное убеждение о стерильности нижних дыхательных путей. Сегодня активно изучают множество доказательств и фактов о выраженных нарушениях микробиома легких при различных респираторных патологиях [11,18,20], в том числе и наличие множества некультивируемых бактерий в жидкости бронхоальвеолярного лаважа (БАЛ) при ИЛФ [13], но состав легочного микробиома при нем остается неизвестным [27]. Соответственно возникает вынужденный поиск оптимальных и точных маркеров для отбора и прогноза, т.к. показатель выживания, по всей вероятности, влияет на приоритетные списки ожидания трансплантации легких больных ИЛФ [25].

В зависимости от предполагаемого их назначения биомаркеры ИФЛ могут быть разные: диагностики, предрасположенности к заболеванию и его прогноза, активности течения и ответа на фармакотерапию. Все эти маркеры изучаются при ИЛФ, однако более разработанными считают биомаркеры прогноза и восприимчивости, например, мутации в белках сурфактанта или укорочение теломер некоторых хромосом. При ИЛФ трудно оценивать активность болезни, поэтому и отсутствует консенсус в вопросе, что именно определяет ее активность. Среди биомаркеров различают оценку генетических факторов и полиморфизм генов, белки крови, рецепторы к метаболическим модификаторам, разные клеточные популяции, участвующие в фиброгенезе -3 все это создает сложную диагностическую мозаику и тем более трудности в эффективной терапии.

В представленном аналитическом обзоре, методы изучения роли биомаркеров базируются на сочетании клинической диагностики и прогноза, при этом наиболее значимым представляется, что в нижеприведенных исследованиях [7,15,39], у большинства больных (70%) диагноз подтвержден открытой биопсией легких, а уровни изучаемых маркеров оценены современными биохимическими, иммуноферментными, статистическими методами, причем биосредами для анализа служили как кровь, так и жидкость БАЛ.

Патогенетическая роль эндотелина-1 в течении ИЛФ. Кроме известных вазоконстрикторных влияний, появились доказательства о роли эндотелина-1 (ЕТ-1) в фиброгенезе: он может быть главной детерминантой хемотаксиса фибробластов (ФБ) в легочной артерии, стимулировать их репликацию, синтез коллагена и уменьшать деградацию коллагена [17,21]. В исследованиях [33,34,41] показано, что экспрессия ЕТ-1 повышена в эпителии легких, в пневмоцитах II типа, эндотелии и в воспалительных клетках.

В исследованиях Barlo N.P. et al. в Department of Pulmonology of the St. Antonius Hospital in Nieuwegein (2010)

уровни ЕТ-1 определяли коммерческими иммуноанализаторами (R&D MN USA) [8]. Значение этого маркера уточнялся рядом исследований у пациентов ИЛФ, и установлен нижний предел его выявления – 0,34 пг/мл в сыворотке крови.

В десятилетний период исследования 1998-2007 г. [8] у 71 пациента ИЛФ (55 мужчин и 16 женщин, средний возраст 62,9 г.) в крови средний уровень ЕТ-1 был достоверно ($p < 0,0001$) повышен до 1,15 пг/мл против контрольной группы 0,85 пг/мл, однако в БАЛ этот уровень маркера был значительно снижен (1,33 пг/мл против 2,10 пг/мл, $p < 0,0005$) [8]. При расчетах соотношения ЕТ-1 с альбумином (Алб) в жидкости БАЛ это снижение еще более очевидно: 16,7 против 63,4, т.е. в 3,8 раза меньше ($p < 0,0001$). Замечена отрицательная корреляция с возрастом больных ИФЛ ($r = -0,36$, $p < 0,007$), однако у здоровых лиц возрастная корреляция в БАЛ не обнаруживалась. Прием низких доз ГК или курение не влияли на уровни ЕТ-1 в обеих биосредах. Клеточные достоверные корреляции уровня ЕТ-1 или ЕТ-1/Алб были установлены у больных, но не у здоровых, только для процентного содержания в жидкости БАЛ макрофагов ($r = +0,34$ и $+0,50$), а не для нейтрофилов, лимфоцитов или эозинофилов. Ассоциаций сывороточных уровней эндотелина с показателями функции внешнего дыхания (ФВД): диффузионной способности легких по СО (DLco), объема форсированного выдоха (FEV1), тотальной легочной емкости (TLC) не обнаружено, хотя ЕТ-1 в БАЛ отрицательно коррелировал со снижением показателя DLco.

Выживаемость больных ИФЛ коррелирует только с соотношением ЕТ-1/Алб в жидкости БАЛ: при разделении пациентов на 2 группы с учетом значения маркера ЕТ-1/Алб до 25 и выше соответственно медиана выживаемости при низком ЕТ-1/Алб достигала 50,4 месяца, в то время как с высоким ЕТ-1/Алб – всего 9 месяцев ($p < 0,006$).

Уточнения различий в уровнях ЕТ-1 в крови и БАЛ, его ассоциации с профибротической ролью макрофагов (МФ) авторы видят в следующем: возможны 2 разных фенотипа МФ – классический активированный МФ, который стимулируется липополисахаридом (ЛПС) или интерфероном IFN- γ (они не влияют на фибробласты), и МФ активированы альтернативным путем (Alternative activation of macrophages – AA-Mac) – через IL-4 или ГК рецепторы. Именно последние фенотипы МФ увеличивают пролиферацию и синтез коллагена ФБ, секрецию TGF- β , тромбоцитарного фактора роста; такой вариант выявлен совсем недавно и мало изучен. Эти результаты авторов [8] о соответствии ЕТ-1 в жидкости БАЛ (но не в крови) прогрессирующему ИЛФ ставят вопрос – достаточно ли значение этого биомаркера для прогноза болезни.

Идеальный биомаркер заболевания должен быть легко доступным, получен неинвазивными методами и специфичен. В то же время, процедура и стандартизация жидкости БАЛ характеризуется изменчивостью концентраций молекул, его клиническая ценность по сей день дискуссионна. Не установлен и состав конденсата выдыхаемого воздуха, что нами было отмечено ранее, т.к. его объемы и состав зависят от ФВД, гемодинамики, obstructивного синдрома, проницаемости аэро-гематического барьера [3]. С учетом вышеизложенного, очевидно, что Barlo N.P. et al. [8] подтверждают концепцию о роли ЕТ-1 в патогенезе ИЛФ, однако не уверены в его прогностической роли.

Необходимо подчеркнуть, что генетические ассоциации биомаркеров более значимы при ИЛФ, чем при других многофакторных хронических заболеваниях. Так, риски генетического полиморфизма, например, для *mus-5b* могут

возрастать в 20 раз [40]. Потому результаты генетического полиморфизма биомаркеров наиболее важны в научных прогнозах.

Роль генетического полиморфизма хемокинов в оценке прогноза у пациентов с ИЛФ. Nicole P. Barlo et al. [10] показали роль провоспалительного СС-Хемокинового лиганда-18 (CCL18) в прогрессировании ИЛФ с позиций его генетических вариаций, что базируется на понимании, что этот лиганд экспрессируется альвеолярными МФ и может достигать сверхвысоких уровней в легких человека, приводя к повышению синтеза ФБ коллагена и молекул экстрацеллюлярного матрикса [4,6,26,31], особенно при альтернативном активированном фенотипе альвеолярных МФ, создавая обратную связь с фибробластами. Кодирующий CCL18 ген невелик, расположен на q плече 17 хромосомы и содержит 3 экзона. Изученная частота носителей аллелей и 3 гаплотипов у пациентов в контроле не отличалась даже в возрастном аспекте [10]. Уровни CCL18 в крови у больных с ИЛФ (645 нг/мл) были достоверно выше в 3,5 раза, чем у здоровых в контроле (185 нг/мл, $p < 0,0001$). В здоровой популяции отмечены определённые различия между носителями и неносителями С-аллеля полиморфизма гена rs2015086: уровни хемокинов при ТТ варианте составили 151 нг/мл, у СТ+СС – 239 нг/мл ($p < 0,0001$). У пациентов с ИЛФ были явные вариации при этом полиморфизме, соответственно уровни CCL18 при ТТ – 585 нг/мл, при СТ – 817 нг/мл ($p < 0,002$); экспрессия мРНК CCL18 положительно коррелирует ($r = +0,73$, $p < 0,002$) с его уровнем в крови. Аллель С характеризовался самой высокой экспрессией мРНК CCL18 как у здоровых, так и у больных.

Пороговое значение CCL18 в площади под кривой (AuC) рассчитано для концентрации 500 нг/мл в крови и для отклонений выше или ниже этого параметра. В результате установлено, что медиана выживаемости при более низком его уровне более оптимальна и достигает 50,4 месяцев, и хуже – только при 27,6 месяцев в группе больных с более высоким уровнем CCL18 ($p < 0,02$). Генотип СТ rs2015086 характеризовался значительно низким показателем выживаемости против генотипа ТТ, соответственно по медиане – 14,3 месяца ($p = 0,01$) [10].

Авторы заключают, что генетическая изменчивость определяет существенные различия мРНК этого лиганда, его уровня в крови и играет модифицирующую роль в течении болезни, что позволяет считать его прогностическим маркером [10], как и при системном склерозе [22].

Роль сурфактантного белка SP-D. Роль сурфактантного белка SP-D оценена исследователями N.P. Barlo et al. – получены доступные результаты у 72 пациентов [7]. Внимание к этому белку сурфактантов уделялось еще в начале 90-х годов [19,30,35]. Авторы определяли концентрации белка с помощью моноклональных антител методом иммуноферментного анализа (ELISA) [7]. У здоровых лиц средние уровни этого белка достигали в сыворотке крови 57,5 нг/мл, без различий по полу, возрасту или факта курения. У больных ИЛФ этот показатель был выше в 6,35 раза, составляя 365 нг/мл ($p < 0,0001$), однако концентрация в жидкости БАЛ, при величине 385 нг/мл, оказалась значительно ниже контроля (504 нг/мл), также вне его зависимости от курения и выживаемости или возраста и без зависимости уровней в БАЛ и крови. Отмечена только отрицательная корреляция SP-D при болезни с параметрами диффузионной способности по монооксиду углерода (rDLco = -0,315, $p < 0,04$).

Поскольку уровень SP-D в сыворотке до 80% зависит от генетического контроля, значимым является полиморфизм кодирующего его гена rs721917: описан аминокислотный полиморфизм в 11 положении, где метионин замещен на треонин (Met11Thr), составляя половину влияний, с последующими изменениями более высокого уровня сурфактантного белка в крови. Несомненно, что генетическая составляющая должна учитываться в оценке прогностических маркеров [35]. Установлено, что частота изученных аллелей у больных и здоровых не отличалась: у первых гомозиготная аллель ТТ гена rs721917 встречалась у 30%, гетерозиготная аллель СТ – у 53% и генотип СС – у 17%, у здоровых соответственно генотип ТТ – 32%, СТ – 52% и СС – 16%, хотя различия в концентрации белка в сыворотке зависели от генотипа у здоровых контролей, а не у больных.

У больных с низкой выживаемостью (менее 6 месяцев) отмечалась обратная зависимость с уровнем этого белка в крови, он был выше (661 нг/мл), чем при длительности жизни 6-12 месяцев (465 нг/мл), а более 12 месяцев еще ниже – 250 нг/мл ($p < 0,0001$), и медиана выживаемости при низком белке составила 50–67 месяцев за критерий разделения принят уровень 460 нг/мл, а при его повышении эта медиана соответствовала только 11 месяцам. Авторы считают, что белок SP-D сыворотки крови может предсказать худший прогноз ИЛФ и смертность (особенно если его уровень выше 460 нг/мл, даже в сроки менее 12 месяцев) и его можно считать новым значимым маркером смертности. Механизм появления SP-D белка в крови, согласно Barlo N.P. et al [7], скорее всего, связан с повышенной проницаемостью аэрогематического барьера и повреждением альвеолоцитов II типа.

Прогностическая роль цитокинов и их генов. Современная концепция ИЛФ предполагает, что ИЛФ развивается при повторяющихся эпизодах повреждения легких на фоне незначительного воспаления, или же болезнь является следствием некоего ремоделирования, однако это требует уточнения [37]. С точки зрения определения роли воспаления в течении и прогнозе ИЛФ, несомненно, важна оценка статуса интерлейкинов, источниками которых могут быть эпителиальные или мезенхимальные клетки, Т- и В-лимфоциты, альвеолярные макрофаги, клетки крови [23]. Значительный интерес представляет определение роли интерлейкина I типа (IL-1), участвующего в формировании фиброза [23]. Семейство IL-1 состоит из трех структурно родственных белков: два из них – IL-1a и IL-1b – агонисты, а третий IL-1Ra – конкурентный антагонист рецептора этого интерлейкина, белок кодируется геном на II хромосоме (IL-1RN) [42]. Бета-интерлейкин-1 (IL-1b) продуцируется активированными альвеолярными МФ и эпителием, стимулируя выработку других цитокинов (TNF-а и IL-6). Полиморфизм гена рецептора определяет чувствительность к ним [32], причем продукция IL-1Ra в МФ выше, чем IL-1b, однако снижение отношения рецептора IL-1Ra к IL-1b способно увеличивать фиброгенез [32].

Акцент на роль интерлейкина в исследовании [9] проведен у 77 пациентов с ИЛФ (средний возраст 60,8 лет), путем сравнения с результатами 349 здоровых добровольцев славянской популяции (средний возраст 39,4 года); показано: уровни IL-1b в сыворотке повышены в сравнении с здоровыми лицами, тогда как уровни рецептора IL-1Ra – снижены. В жидкости БАЛ оба они значительно повышены, еще более повышены у больных, принимавших препараты глюкокортикоиды.

Соотношение в БАЛ IL-1Ra к IL-1b (215,7 против 771,4 в контроле), очевидно, в 3,5 раза ниже, с аналогичными изменениями в сыворотке крови (77,9 против 293,5, $p < 0,0001$). В крови гормоны изменяли это соотношение в сторону повышения: 101,7 против 71,5 ($p < 0,01$).

Что касается полиморфизма гена рецептора IL-1Ra или IL-1b, то он не влияет на уровни интерлейкинов ни в крови, ни в БАЛ [9]. Но перенос аллеля G в гене IL-1Ra rs2637988 более тесно связан с ИЛФ: его носительство у больных выше – 75% против контроля 61%, ($p < 0,02$) и сочетается с более низким соотношением IL-1Ra/IL-1b в БАЛ, т.е. имеет место относительный дефицит IL-1Ra в сравнении с IL-1b, означая, что именно присутствие G-аллеля играет патогенетическую роль. Повышение уровня этого рецептора в БАЛ было недостаточным, чтобы сравниться с огромным увеличением местного синтеза IL-1, что ведет к снижению IL-1Ra/IL-1b против контроля. Известно, что сверхвысокие уровни IL-1b в легких блеомициновых крыс сопровождалась тяжелым фиброзом, скоплением миофибробластов, очагами появления коллагена и фибронектина [23], в то время как введение экзогенного рецептора IL-1Ra предотвращает эти процессы. Такие результаты позволяют судить о положительной роли гена IL-1Ra в предрасположенности и начальном запуске патологического процесса. Баланс между про- и противовоспалительными цитокинами имеет более значимое биологическое значение, чем их абсолютные концентрации [9].

Ограничение значения полученных результатов

Приведенные выше данные касательно некоторых биомаркеров указывают на сложность проблемы с точки зрения прогноза ИЛФ. Разногласия в оценке этих исследований касаются, прежде всего, избираемых конечных точек, они разные и часто недостаточно обоснованные, однако следует подчеркнуть, что такие существенные подходы к первичным и вторичным конечным точкам, особенно в прогнозировании смертности, остаются предметом продолжительных дискуссий. Очевидно, что для оценки прогноза смертности необходимо включать очень большое количество пациентов, продлевать сроки наблюдения, что увеличивает их стоимость и снижает возможности и желание фармацевтических компаний участвовать в этих исследованиях. Предложенный подход к оценке роли ФЖЕЛ в динамике для прогнозов ИЛФ требует количественного уточнения, тем более в условиях значительной клинической гетерогенности этого заболевания [5].

В проанализированных источниках научной литературы недостаточно четко отражены клинические и морфологические варианты болезни, однако предлагается несколько классификационных разновидностей течения ИЛФ: интерстициальная пневмония, десквамативная пневмония, болезнь Хаммана-Рича и неспецифическая интерстициальная пневмония с гистологическими отличиями.

Тем не менее, в последнее десятилетие нарастающее число различных дизайнов клинических исследований по ИЛФ послужит уточнению прогноза и появлению корректных фармакологических подходов к его лечению [1,2,5]. Очевидно, что прогрессирующее накопление в легких фибробласт-миофибробластных очагов при ИЛФ ухудшает прогноз, формирует собственную среду с набором цитокинов, ростовых факторов, коллагена, фибронектина во внеклеточном матриксе фиброзных легких (фибриновая подложка), особенно в их различных сочетаниях и изменяющемся балансе между факторами как пато-, так и саногенеза. Сложности решения успешной фармакотерапии базируются на

этих разнородных клеточных популяциях в легких, они тем более «запутываются» и дискутируются в условиях эпителиально-мезенхимального перехода, когда (почти 50%) альвеолоциты могут трансформироваться в фибробласты и подобные им клетки. Эти условия реализуются также при активации мультипотентных эпителиальных стволовых или прогениторных клеток.

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SUMMARY

PROGNOSTIC VALUE OF BLOOD AND BRONCHOALVEOLAR LAVAGE FLUIDS BIOMARKERS FOR IDIOPATHIC PULMONARY FIBROSIS (REVIEW)

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The article reveals the modern aspects of IPF pathogenesis in with an emphasis on the main proposed prognostic biomarkers. IPF remains the leader among diseases with unknown etiology, the diagnosis and management of which are not very successful, despite the obvious progress in molecular medicine. There is presented analysis of the significance of IPF potential biomarkers and their concentrations in the blood and bronchoalveolar lavage fluids (BAL): endothelin-1, CC-chemokine ligand 18, interleukin-1, surfactant protein SP-D in the review. The role of their changing levels in the blood and BAL for assessing the course of the IPF and its prognosis, as well as the prevailing importance of the polymorphism of the genes encoding them, is shown. Obviously, the progressive accumulation of fibroblast-myofibroblast cells in the lungs IPF patients worsens the prognosis of disease, forms its own environment with a set of cytokines, growth factors, collagen, fibronectin in the extracellular matrix of fibrous lungs. The insufficient amount of studies in the face of the rarity of the disease leaves a lot of controversial issues for solution in the future. Obviously, to assess the prognosis of IPF mortality, it is necessary to include a very large number of patients, to extend the observation period, which increases their cost and reduces the opportunities and desire of pharmaceutical companies to participate in these studies.

Keywords: idiopathic pulmonary fibrosis (IPF), bronchoalveolar lavage fluids (BAL), endothelin-1, CC-chemokine ligand 18, interleukin-1, surfactant protein SP-D.

РЕЗЮМЕ

ПРОГНОСТИЧЕСКОЕ ЗНАЧЕНИЕ БИОМАРКЕРОВ КРОВИ И БРОНХОАЛЬВЕОЛЯРНОГО ЛАВАЖА ДЛЯ ИДИОПАТИЧЕСКОГО ЛЕГОЧНОГО ФИБРОЗА (ОБЗОР)

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В статье рассмотрены современные аспекты патогенеза ИЛФ с акцентом на основные предлагаемые прогностические маркеры. ИЛФ остается лидером среди заболеваний с неизвестной этиологией, диагностика и лечение которых, несмотря на очевидный прогресс молекулярной медицины, малоуспешны. Представлен анализ значимости потенциальных маркеров ИЛФ и их концентраций в крови и жидкости бронхоальвеолярного лаважа: эндотелина-1, СС-Хемокинового лиганда 18, интерлейкина-1, сурфактантного белка SP-D. Показана роль их изменяющихся уровней в обеих биосредах для оценки течения болезни и ее прогноза, а также превалирующее значение полиморфизма кодирующих их генов. Очевидно, что прогрессирующее накопление в легких фибробласт-миофибробластных очагов при ИЛФ ухудшает прогноз, формирует собственную среду с набором цитокинов, ростовых факторов, коллагена, фибронектина во внеклеточном матриксе фиброзных легких. Недостаточное количество исследований в условиях редкости болезни оставляет ряд дискуссионных вопросов для решения в будущем. Очевидно, что для оценки прогноза смертности необходимо включать большое количество пациентов, продлить сроки наблюдения, что увеличит их стоимость, снизит возможности и желание фармацевтических компаний участвовать в этих исследованиях.

რეზიუმე

სისხლის და ბრონქოალვეოლური ლავაჟის ბიომარკერების პროგნოზული მნიშვნელობა ფილტვის იდიოპათიური ფიბროზის დროს (მიმოხილვა)

ო. იაკოვლევა, ა. კლკოტი, ნ. შჩერბენიუკი,
ო. გოინა-კარდასევიჩი

ვინიცას ნ.პიროგოვის სახელობის ეროვნული სამედიცინო უნივერსიტეტი, უკრაინა

სტატიაში განხილულია ფილტვის იდიოპათიური ფიბროზის პათოგენეზის თანამედროვე ასპექტები, აქცენტით ძირითად პროგნოზულ მარკერებზე. ფილტვის იდიოპათიური ფიბროზი ლიდერად რჩება უცნობი ეტიოლოგიის დაავადებათა შორის, რომელთა მკურნალობა ნაკლებშედეგიანია, მიუხედავად თვალსაჩინო პროგრესისა მოლეკულური მედიცინის სფეროში. ნაშრომში წარმოდგენილია ფილტვის იდიოპათიური ფიბროზის თანამედროვე ასპექტები, აქცენტით ძირითად პროგნოზულ მარკერებზე. ფილტვის იდიოპათიური ფიბროზი ლიდერად რჩება უცნობი ეტიოლოგიის დაავადებათა შორის, რომელთა მკურნალობა ნაკლებშედეგიანია, მიუხედავად თვალსაჩინო პროგრესისა მოლეკულური მედიცინის სფეროში. ნაშრომში წარმოდგენილია ფილტვის იდიოპათიური ფიბროზის პათოგენეზის თანამედროვე ასპექტები, აქცენტით ძირითად პროგნოზულ მარკერებზე. ფილტვის იდიოპათიური ფიბროზი ლიდერად რჩება უცნობი ეტიოლოგიის დაავადებათა შორის, რომელთა მკურნალობა ნაკლებშედეგიანია, მიუხედავად თვალსაჩინო პროგრესისა მოლეკულური მედიცინის სფეროში.

თიური ფიბროზის პოტენციური მარკერების და მათი კონცენტრაციის მნიშვნელობის ანალიზი სისხლსა და ბრონქოალვეოლურ ლავაჟში: ენდოთელინი-1, ქემოკინური ლიგანდი-18, ინტერლეიკინი-1, სურფაქტანტური ცილა SP-D. ნაჩვენებია მათი ცვალებადი დონის მნიშვნელობა ორივე გარემოში დაავადების მიმდინარეობისა და პროგნოზისათვის, ასევე, მათი გენების პოლიმორფიზმის მაკოდირებელი მნიშვნელოვანი და უპირატესი როლი. აშკარაა, რომ ფიბრობლასტ-მიოფიბრობლასტური კერების პროგრესირებადი დაგროვება ფილტვებში ფილტვის იდიოპათიური ფიბროზის დროს აუარესებს დაავადების პროგნოზს, ფიბროზული

ფილტვის უჯრედშორის მატრიქსში აყალიბებს საკუთარ გარემოს ციტოკინების, ზრდის ფაქტორების, კოლაგენის და ფიბრონექტინის ნაკრებით.

კვლევების არასაკმარისი რაოდენობა ამ იშვიათი დაავადების დროს ტოვებს მომავალში გადასაწყვეტ რიგ სადისკუსიო საკითხებს. ნათელია, რომ სიკვდილობის პროგნოზის შეფასებისათვის საჭიროა პაციენტების დიდი რაოდენობა და დაკვირვების პერიოდის გახანგრძლივება, რაც ზრდის კვლევის ღირებულებას და ამცირებს ფარმაცევტული კომპანიების შესაძლებლობებს და სურვილს ამ კვლევებში მონაწილეობისათვის.

МОЛЕКУЛЯРНО-ГЕНЕТИЧЕСКИЕ ОСОБЕННОСТИ ТЕЧЕНИЯ ТЯЖЕЛЫХ ФОРМ ТРОПИЧЕСКОЙ МАЛЯРИИ (ОБЗОР)

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Малярия является одной из значимых проблем мирового здравоохранения. Согласно данным Всемирной организации здравоохранения, в 2017 г. в мире зарегистрировано 219 млн. случаев заболевания малярией, 435 000 летальных исходов, преимущественно, в странах Африки и Юго-Восточной Азии. Риск заболеть малярией подвергается 3.2 млрд. человек в мире [32].

Несмотря на то, что к началу 1960 г. малярия в СС была практически полностью ликвидирована, в 1990 г., в связи с ухудшением социально-экономической ситуации, очаги малярии вновь образовались на территории бывших союзных республик и на юге РФ (*P.falciparum* – Таджикистан; *P.vivax* – Азербайджан, Таджикистан; *P.malariae* – Закавказье, Средняя Азия), из этих стран вследствие массовой сезонной миграции рабочих заболевание распространилось на всю территорию РФ и стран СНГ [3]. Рост случаев заболеваемости завозной малярией связан также с расширением международных связей со странами Африки, Юго-Восточной Азии, Латинской Америки [4]; развитием международного туризма.

Ежегодно на территории РФ регистрируется около 100 случаев заболеваемости малярией. Большинство из них приходится на тропическую малярию - 73% в 2017 г.

В таблице 1 и на рис. 1 представлены данные о возбудителях малярии и случаях заболеваемости в РФ за 2013-2017 гг. [7–11].

Тропическая малярия характеризуется самым тяжелым течением и высокой смертностью (до 98% всех летальных исходов). Наиболее частыми осложнениями являются церебральная форма малярии, тропическая алгидная малярия, гемоглобинурийная лихорадка, острая почечная недостаточность, нефротический синдром, респираторный дистресс-синдром. Церебральная форма малярии протекает максимально тяжело, развивается в 10% всех случаев тропи-

ческой малярии в мире; 60-80% летальных исходов малярии вызвано развитием церебральной формы [1]. В 2013-2017 гг. в Российской Федерации зарегистрировано 9 случаев заболеваемости тропической малярией с летальным исходом, 8 из них в результате ее церебральной формы [5].

Церебральная форма малярии (малярийная кома, инфекционно-токсическая энцефалопатия) является осложнением тропической малярии, характеризуется тяжелыми неврологическими нарушениями (кома-статус - ШКГ <11, шкала ком Blantyre <3); судорогами с сохранением коматозного статуса более 30 минут [31]. Несмотря на то, что церебральная форма малярии практически всегда вызвана *P.falciparum*, известны отдельные случаи тяжелых неврологических осложнений в результате инвазии *P.vivax* и *P.knowlesi* (без развития комы) [13,17,25].

Клиническая картина

Церебральная форма малярии наиболее часто развивается у неиммунных лиц на I-II неделе болезни, в основном, в результате запоздалого или неадекватного лечения [1]. Возможно более раннее развитие церебральной формы малярии; ее проявления в течение первых 23-48 часов отмечаются преимущественно у больных с дефицитом массы тела [4].

Церебральная форма малярии развивается стремительно. В случае отсутствия своевременного и адекватного лечения смерть наступает в течение 1-5 суток после появления первых симптомов [2].

При описании коматозных состояний принято использовать шкалу комы Глазго (1974 г.) или ее модифицированную версию - шкалу FOUR.

Выделяют 3 стадии развития заболевания: (1) сомноленция; (2) сопор; (3) кома. Развитию церебральной формы малярии предшествуют сильная головная боль, резкая слабость; больные апатичны либо, возбуждены [1].

Таблица 1. Заболеваемость малярией на территории РФ за 2013-2017 гг.

Год	Возбудитель	Кол-во больных, чел.	Долевое участие
2013	<i>P.falciparum</i>	44	0.46
	<i>P.vivax</i>	43	0.45
	<i>P.ovale</i>	7	0.07
	<i>P.malariae</i>	1	0.01
	mixt (<i>P.falciparum</i> + <i>P.ovale</i>)	4	0.04
	Итого	95	1.00
2014	<i>P.falciparum</i>	48	0.48
	<i>P.vivax</i>	46	0.46
	<i>P.ovale</i>	6	0.06
	<i>P.malariae</i>	1	0.01
	mixt	0	0
	Итого	101	1.00
2015	<i>P.falciparum</i>	57	0.58
	<i>P.vivax</i>	37	0.37
	<i>P.ovale</i>	3	0.03
	<i>P.malariae</i>	0	0
	mixt (<i>P.falciparum</i> + <i>P.ovale</i>)	1	0.01
	Итого	99	1.00
2016	<i>P.falciparum</i>	64	0.63
	<i>P.vivax</i>	31	0.31
	<i>P.ovale</i>	6	0.06
	<i>P.malariae</i>	1	0.01
	mixt	0	0
	Итого	101	1.00
2017	<i>P.falciparum</i>	68	0.73
	<i>P.vivax</i>	23	0.25
	<i>P.ovale</i>	1	0.01
	<i>P.malariae</i>	1	0.01
	mixt	0	0
	Итого	93	1.00

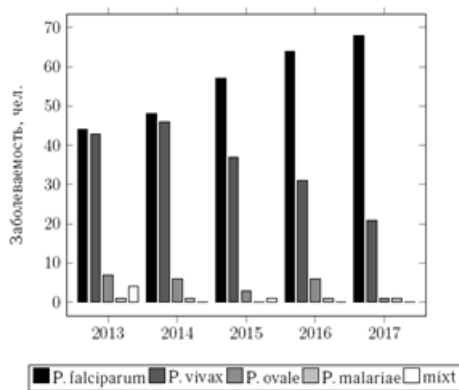


Рис. 1. Заболеваемость малярией на территории РФ за 2013-2017 гг.

1. Сомноленция (прекома). Отмечаются незначительные нарушения психики и сознания, снижение двигательной ак-

тивности, психическое истощение. Больной отвечает на вопросы неохотно, односложно, быстро возвращается в состояние дремы. Характерная поза: голова запрокинута назад, ноги разогнуты, руки согнуты в локтевых суставах. Менингеальные симптомы обычно положительные (ригидность затылочных мышц, симптомы Кернига, Брудзинского), вызваны поражением нервных центров, обеспечивающих мышечный тонус, и кровоизлияниями в оболочки мозга. Иногда наблюдается гиперкинез: тонические судороги мышц туловища и конечностей, тетанические и эпилептиформные судорожные приступы. Сухожильные рефлексы, как правило, нормальные.

В периферической крови высокая паразитемия (50 тыс. в мкл и больше), нейтрофильный лейкоцитоз, температура тела фебрильная либо пиретическая (38.5–40.5°C), АД снижено (90/50–80/40 мм рт. ст.), пульс соответствует температуре (учащен), тоны сердца приглушены. Наблюдается тахипноэ, частота дыхательных движений (ЧДД) 30–50/мин, дыхание поверхностное. Печень и селезенка увеличены,

при пальпации плотные. Кожа и слизистые бледные, субиктеричные. Возможны точечные кровоизлияния.

Могут наблюдаться симптомы выраженного токсического гепатита, отека легких, олигурии, тяжелой анемии (Hct<0.2л/л, Hgb=60 г/л).

2. *Сонор.* Развиваются существенные нарушения психики и сознания: спутанность сознания, дезориентация во времени и пространстве, состояние сонливости, из которого больного удается вывести только болевым раздражением или окриком. Сухожильные рефлексы повышаются до степени клонуса. Глотательный и роговичный рефлексы сохранены. Реакция зрачков на свет вялая. Возникают непроизвольные мочеиспускание и дефекация.

3. *Кома.* Наблюдается полная утрата сознания. Больной не реагирует на внешние раздражители. Исчезают сухожильные рефлексы: вначале – глоточный, затем – роговичный и зрачковый. Постепенно исчезают брюшные и крематорные рефлексы. Появляется симптом «плавающих глазных яблок». Развивается тахикардия, одышка (ЧДД 40–60/мин). В агональном периоде присоединяется отек легких. В периферической крови нейтрофильный лейкоцитоз, СОЭ повышено до 60 мм/ч [3].

Патогенез

В основе развития церебральной формы малярии лежат 3 фактора:

1) секвестрация пораженных эритроцитов, обусловленная их адгезией к клеткам эндотелия, в т.ч. к эндотелию микроциркуляторного русла головного мозга, и т.н. розеткообразование – адгезия пораженных эритроцитов к неповрежденным;

2) воспаление, вызванное повышением уровня цитокинов, и, как следствие, агрегацией лейкоцитов в сосудах головного мозга;

3) нарушение целостности гематоэнцефалического барьера [19].

Кроме того, неспецифичными для церебральной малярии факторами являются анемия, вызванная гемолизом пораженных эритроцитов и дефицитом железа из-за образования гемозоина, а также диссеминированное внутрисосудистое свертывание (ДВС-синдром), приводящее к местному замедлению микроциркуляции крови. Оба этих фактора приводят к гипоксии, вплоть до аноксии, в т.ч. тканей головного мозга [2,33].

Секвестрация пораженных эритроцитов вызвана связыванием антигенов (лигандов), экспрессируемых плазмодием на поверхность эритроцитов, с рецепторами эндотелиоцитов микроциркуляторного русла головного мозга. Дальнейшее розеткообразование, аналогичным образом, обусловлено связыванием лигандов на поверхности пораженных эритроцитов с белками неповрежденных эритроцитов. К числу белков, экспрессируемых *P.falciparum*, вызывающих клеточную адгезию, относятся семейства белков PfEMP1, RIFIN, STEVOR. Потенциальными лигандами являются также другие белки, выделяемые плазмодием на поверхность эритроцита: SURFIN (surface-associated interspersed), PfMC-2TM (*P.falciparum* Maurer's cleft two transmembrane), видоизмененный белок полосы 3 [15].

Наиболее хорошо изученным является семейство белков PfEMP1 (*P.falciparum* erythrocyte membrane protein 1, *P.falciparum* эритроцитарный мембранный белок 1). Белки данного семейства являются высокомолекулярными соединениями, которые кодируются семейством генов *var*, в состав которого входят около 60 генов. Общая структу-

ра молекулы PfEMP1 выглядит следующим образом: белок кодируется 2 экзонами. Экзон 1 кодирует домены, расположенные на N-конце белка: N-концевой сегмент (N-terminal segment, NTS), перемежающиеся домены - Duffy-binding-like (DBL) и cysteine-rich interdomain region (CIDR), трансмембранный домен (transmembrane domain, TMD). Участки экзона 1 отличаются высокой вариабельностью. Экзоном 2 кодируется кислотный концевой сегмент (acid terminal segment, ATS), расположенный на C-конце белка [20,26]. Принципиальная схема строения белка PfEMP1 приведена на рис. 2. Единственно в пораженном эритроците имеется единственный вариант белка PfEMP1 [33].

На поверхности пораженного эритроцита PfEMP1 расположен на мембранных бугорках, образующихся через некоторое время после инвазии. N-концевой участок белка обращен наружу, а C-концевой – внутрь эритроцита. Посредством белков PHIST (plasmodium helical intersperse subtelomeric) и KAHRP (knob-associated histidine-rich protein) C-концевой участок связан с цитоскелетом пораженного эритроцита (спектрином, актином, анкирином R и комплексом спектрин-актин-белок полосы 4.1) [21].

PfEMP1 способен связываться с большим количеством рецепторов: ICAM-1 (intercellular adhesion molecule-1/CD54, молекула межклеточной адгезии-1), CR1, CD36, CD31, гепарансульфатом (heparane sulphate, HS), хондроитинсульфатом A (chondroitine sulphate A, CSA) и т.д. В патогенезе церебральной малярии наибольшее значение имеет связывание PfEMP1 с рецепторами эндотелия, обеспечивающее секвестрацию эритроцитов. Таковыми являются CD36, EPCR (endothelial protein C receptor, эндотелиальный рецептор протеина C), HS, тромбоспондин (thrombospondin, TSP), P-селектин и, в особенности, ICAM-1.

Отдельно следует рассматривать связывание PfEMP1 с рецептором EPCR, осуществляемое посредством кассеты доменов 8 участка CIDR1 α . В норме с EPCR связывается протеин C, и происходит его активация. Активированный протеин C оказывает антикоагулянтное, противовоспалительное, цитопротективное действие. В результате конкуренции с PfEMP1 за связывание с EPCR, количество активированного протеина C уменьшается, происходит адгезия пораженных эритроцитов к клеткам эндотелия [28, 29].

Белки семейства PfEMP1 также способствуют розеткообразованию, связываясь с молекулами CR1 (complement receptor 1, рецептор комплемента 1) и с гепарансульфатом на поверхности неинвазированных эритроцитов посредством домена NTS-DBL1 α 1. Адгезия посредством гепарансульфата более слабая, и ингибируется гепарином [33]. Также возможно связывание пораженных эритроцитов с помощью α 2-макроглобулинов, находящихся на их поверхности; адгезия усиливается в присутствии молекул иммуноглобулина M (IgM) [27].

Секвестрация пораженных эритроцитов препятствует их удалению селезенкой, в результате предшествует розеткообразованию. Возможно одновременное участие одной молекулы PfEMP1 как в секвестрации, так и в розеткообразовании, обусловленное, вероятно, одновременным связыванием лигандов с доменами NTS-DBL1 α и DBL2 γ . Помимо антигенов семейства PfEMP1, трофозоиты и эритроцитарные шизонты *P.falciparum* экспрессируют антигены семейства RIFIN (repetitive interspersed family), способные участвовать в розеткообразовании. Данные белки кодируются вариабельным семейством генов *rifin*, подразделяющимся на 2 подсемейства: *rifA* и *rifB*. Аналогичным

образом, белки подразделяются на 2 типа, тип А и тип В, в зависимости от того, генами какого из подсемейств они кодируются. Различия между типами выражается, в частности в локализации белков. Белки RIFIN типа А расположены на поверхности мембраны эритроцита, куда они транспортируются с помощью т.н. пятен Маурера, а также на апикальном конце мерозоида. RIFIN типа В присутствует в цитоплазме мерозоитов. Белки семейства RIFIN экспрессируются на поверхности пораженного эритроцита одновременно с белками семейства PfEMP1. Они связываются преимущественно с агглютиногенами группы А, образуя крупные розетки. Кроме того, более мелкие розетки образуются ввиду того, что лиганды RIFIN способны связываться с эритроцитами группы 0 за счет связывания с гликофорин-ом А, расположенным на поверхности эритроцитов. Белки семейства RIFIN типа А, по всей вероятности, служат препятствием для распознавания находящихся на поверхности пораженного эритроцита белков семейства PfEMP1 иммунными клетками [14, 33].

Антигены семейства STEVOR (subtelomeric variable open reading frame) расположены на поверхности пораженных эритроцитов у основания выпуклостей. Они кодируются генами семейства *stevor*, насчитывающего 33 гена, также отличающихся высокой вариабельностью. Экспрессия STEVOR начинается чуть позже, чем экспрессия PfEMP1 и RIFIN, на стадии взрослого трофозоида. Белки данного семейства связываются с гликофорин-ом С на поверхности непораженных эритроцитов, способствуя также розеткообразованию. В отличие от PfEMP1, способного к образованию розеток лишь в посткапиллярных венулах, где кровяное давление низкое, STEVOR образует более прочные связи. Вместе с тем, данный белок приводит к увеличению жесткости мембраны эритроцита, вследствие чего изменяются реологические свойства крови. Белки семейства STEVOR, подобно белкам семейства RIFIN, могут находиться и на поверхности мерозоитов. Учитывая время начала экспрессии, локализацию и высокую вариабельность, вероятным предназначением белков семейства STEVOR является защита мерозоитов от гуморального иммунного ответа [18,23,33].

Таким образом, секвестрация эритроцитов начинается с экспрессии антигена PfEMP1 на поверхность пораженных эритроцитов, за счет чего происходит их адгезия к клеткам эндотелия сосудов; происходит розеткообразование вокруг указанных эритроцитов, однако роль PfEMP1 в этом процессе вторична. Одновременно с PfEMP1 на поверхность эритроцита экспрессируется RIFIN типа А, а чуть позже – STEVOR, усиливающие процесс розеткообразования. В результате происходит обструкция сосудов, развивается ишемия, гипоперфузия тканей головного мозга, приводящая к гипоксии и развивающейся вследствие этого церебральной патологии [33].

Кроме того, для церебральной формы малярии также характерна адгезия эритроцитов, опосредованная тромбоцитами, при которой наблюдается явление аутоагглютинации [16]. Данное явление обусловлено связыванием антигенов плазмодия с лигандами CD36, а также Р-селектином и gC1qR, причем наличие белка CD36 является необходимым условием адгезии [16,22,24,30].

Другим значимым патогенетическим фактором церебральной формы малярии является воспалительный иммунный ответ, вызванный наличием чужеродных молекул на поверхности пораженных эритроцитов и, в небольшой степени, находящихся в кровеносном русле мерозоитов.

Клетки системы врожденного иммунитета, например, макрофаги и дендритные клетки, а также клетки эндотелия и фибробласты, содержат на своей поверхности т.н. паттерн-распознающие рецепторы (pattern-recognising receptors, PRR), в частности, TLR, С-лектины, RIG-подобные и NOD-подобные рецепторы. В результате воздействия чужеродных патоген-ассоциированных молекулярных паттернов (pathogen-associated molecular pattern, PAMP) происходит активация PRR, в первую очередь, TLR. Последние активируют сигнальные пути факторов транскрипции NF- κ B, AP-1 и интерфероновых регуляторных факторов, за счет чего начинается синтез и экспрессия провоспалительных цитокинов: интерферона γ (IFN γ), интерлейкинов IL-6 и IL-12, фактора некроза опухолей (tumor necrosis factor, TNF) и т.д. [12] Отдельно следует отметить значимость фактора некроза опухолей в патогенезе церебральной малярии, поскольку его воздействие на эндотелиоциты приводит к повышению экспрессии на поверхности последних молекул клеточной адгезии, таких как ICAM-1 и VCAM-1, что приводит к усилению секвестрации эритроцитов. При церебральной форме малярии наибольшая экспрессия ICAM-1 наблюдается в микроциркуляторном русле головного мозга. Помимо этого, под действием провоспалительных цитокинов клетки эндотелия способны самостоятельно выделять как про-, так и противовоспалительные цитокины, а также хемокины. Повышение уровня хемокинов в головном мозге приводит к хемотаксису лейкоцитов (макрофагов, моноцитов, нейтрофилов, Т лимфоцитов), усиливая воспаление. Вновь поступившие в сосуды головного мозга лейкоциты, в свою очередь, также продуцируют цитокины и хемокины. Поскольку при тропической малярии происходит также секвестрация лейкоцитов, хемотаксис большого количества лейкоцитов приводит не только к усилению воспалительного иммунного ответа, но и к агрегации лейкоцитов в МЦР головного мозга.

Вследствие перераспределения белков, образующих плотные контакты (окклюдина, винкулина, zonula occludens-1 (ZO-1)), под действием цитокина MCP-1/CCL2, вызывающего снижение экспрессии кавеолина-1, происходит разрушение межклеточных соединений и снижение устойчивости эндотелия, вызывающее нарушение целостности гематоэнцефалического барьера в местах секвестрации пораженных эритроцитов. Пораженные эритроциты выделяют везикулы, содержащие микроРНК miR-451a; происходит эндоцитоз везикул эндотелиоцитами, также приводящий к снижению экспрессии ими кавеолина-1. Сходным образом, при воздействии TNF и IFN γ в эндотелиоцитах головного мозга повышается количество микроРНК miRNA-155, что приводит к снижению количества клаудина-1 в плотных контактах и, как следствие, перераспределению белка ZO-1, вызывающему повышение проницаемости ГЭБ [19].

В результате нарушения целостности гематоэнцефалического барьера происходит попадание цитокинов и продуктов жизнедеятельности плазмодия в паренхиму головного мозга; активация астроцитов и микроглии, в результате чего они начинают экспрессировать хемокины, вызывая хемотаксис лейкоцитов к головному мозгу; их дальнейшая секвестрация приводит к воспалению. Одновременно, ввиду повреждения гематоэнцефалического барьера происходит излитие жидкости из сосудов в внеклеточное пространство головного мозга, приводящее к его вазогенному отеку, появлению геморрагий и очаговых некрозов; образуются т.н. гранулемы Дюрка [6]. Кроме того, активированные клетки

микроглии выделяют лиганд FasL, который, связываясь с экспрессируемым на поверхности активированных астроцитов белком Fas, может приводить к повреждению астроцитов и, как следствие, нарушению функционирования нейронов, влекущему за собой тяжелые неврологические проявления церебральной формы малярии [19].

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SUMMARY

SEVERE FORMS OF TROPICAL MALARIA: MOLECULAR GENETIC FEATURES OF THE COURSE OF THE INFECTION (REVIEW)

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The article overviews some issues of the severe course of tropical malaria. In addition to the analysis of the ongoing situation with malaria in Russia, a general clinical picture of the severe course of tropical malaria is discussed. The main part of the overview includes a detailed analysis of current data on the molecular genetic aspects of the erythrocytes' adhesion in the case of tropical malaria. The main elements involved in the process of binding red blood cells and, as a result, in the process of their adhesion to other cells of the human body were considered in detail. Data were studied and summarized not only on protein interactions between an infected red cell and its cellular environment, but also on the genetic characteristics of the parasite leading to similar molecular-biological processes. In addition to the study of protein PfEMP1 role which is nowadays well-considered in the literature, the most up-to-date but less reported data on erythrocyte adhesion proteins STEVOR and RIFIN were also included. The team of authors hopes that this publication will help to get a deeper insight into the problem of erythrocyte adhesion in the course of complicated malaria infection forms and to summarize some of the available data on this issue.

Keywords: malaria, tropical malaria, P. falciparum, red blood cell adhesion.

РЕЗЮМЕ

МОЛЕКУЛЯРНО-ГЕНЕТИЧЕСКИЕ ОСОБЕННОСТИ ТЕЧЕНИЯ ТЯЖЕЛЫХ ФОРМ ТРОПИЧЕСКОЙ МАЛЯРИИ (ОБЗОР)

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В статье представлен обзор современных данных по проблематике осложненного течения тропической малярии. Помимо актуальных данных по заболеваемости малярией в России, представлена общая клиническая картина осложнений тропической малярии. Основная часть работы содержит анализ актуальных данных по молекулярно-ге-

нетическим особенностям адгезии эритроцитов в течении тропической малярии. Подробно рассмотрены основные элементы, вовлеченные в процесс связывания эритроцитов и, как следствие, их адгезии к другим клеткам организма человека. Изучены и обобщены данные не только о белковых взаимодействиях между инфицированным эритроцитом и его клеточным окружением, но и генетические особенности паразита, приводящие к подобным молекулярно-биологическим процессам. Помимо роли белка PfEMP1 в патогенезе тропической малярии, изучены современные данные о таких белках эритроцитарной адгезии как STEVOR и RIFIN, роли которых уделено недостаточно внимания. Авторский коллектив надеется, что данная публикация поможет более полно взглянуть на проблему эритроцитарной адгезии в процессе протекания осложненных форм малярийной инфекции и обобщить некоторые имеющиеся данные по указанной проблематике.

რეზიუმე

ტროპიკული მალარიის მიმე ფორმების მიმდინარეობის მოლეკულურ-გენეტიკური თავისებურებები (მიმოხილვა)

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მოსკოვის ი.სეჩენოვის სახ. პირველი სახელმწიფო სამედიცინო უნივერსიტეტი, რუსეთის ფედერაცია

სტატიაში წარმოადგენილია თანამედროვე მონაცემების მიმოხილვა ტროპიკული მალარიის გართულებული მიმდინარეობის პრობლემატიკასთან დაკავშირებით. რუსეთში მალარიით ავადობის აქტუალური მონაცემების გარდა, წარმოადგენილია ტროპიკული მალარიის გართულებების კლინიკური სურათი. ნაშრომის ძირითადი ნაწილი მოიცავს თრომბოციტების ადჰეზიის მოლეკულურ-გენეტიკური თავისებურებების აქტუალური მონაცემების ანალიზს ტროპიკული მალარიის მიმდინარეობისას. დაწვრილებითაა განხილული ერითროციტების დაკავშირების პროცესში ჩართული ძირითადი ელემენტები და, როგორც შედეგი, მათი ადჰეზია ადამიანის ორგანიზმის სხვა უჯრედებთან. შესწავლილი და განხილულია მონაცემები არამარტო ცილოვანი ურთიერთქმედებების შესახებ ინფიცირებულ ერითროციტსა და მის უჯრედულ გარემოცვას შორის, არამედ პარაზიტის გენეტიკური თავისებურებებიც, რომელიც ამგვარ მოლეკულურ-ბიოლოგიურ პროცესებს იწვევს. ტროპიკული მალარიის პითოგენეზისში PfEMP1 ცილის როლის გარდა, შესწავლილია მონაცემები ერითროციტული ადჰეზიის გამომწვევი ცილების – STEVOR- და RIFIN-ის შესახებ, რომელთა როლს არასაკმარისი ყურადღება ექცევა. ავტორთა კოლექტივი იმედოვნებს, რომ წინამდებარე პუბლიკაცია ხელს შეუწყობს ერთოციტული ადჰეზიის პრობლემის უფრო სრულად დანახვას მალარიული ინფექციის გართულებული ფორმების მიმდინარეობისას და ამ პრობლემის ირგვლივ არსებული მონაცემების განხილვას.

IMPORTED TROPICAL MALARIA (CASE REPORT)

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Malaria is a parasitic disease. It is one of blood infections caused by malaria plasmodia. The disease is transmitted to a human by a bite of a female mosquito of *Anopheles* genus. A cyclic course followed by episodic attacks of fever and periods of epirexia, enlargement of liver and/or spleen, anaemia, sometimes severe disorders of the nervous system, kidneys and other organs, as well as the tendency towards relapses are characteristic features of it.

In 2017, 219 million people suffered from this disease worldwide, more than 3 million died [1,11]. According to the WHO, as a result of implementation of global malaria prevention programs in 2013, global mortality rates have decreased by 47% compare to 2000, and by 54% in Africa [2]. The children under 5 years of age are the risk group of the disease and death from it [3].

According to the WHO experts, about half of the world population (3.2 billion) is at risk of this dangerous invasion at present [4]. In 2015, 214 million cases of malaria caused by five established pathogens were registered: *Plasmodium vivax* that causes three-day malaria, *P. ovale* – ovale malaria, *P. malariae* – four-day malaria, *P. falciparum* – tropical malaria and *P. knowlesi* – knowlesi malaria [5]. Most of the sick (89%) are indigenous people of Africa [6]. In the USSR there were three major outbreaks of the disease (1923-1924, 1934-1935, and 1944-1945); since 1964 only random cases of malaria have been registered [7].

Local malaria transmission in Ukraine has not been registered since 1956, however, every year some imported cases occur, mainly in foreigners, who come to study from the countries affected by this disease: Angola, Ethiopia, Egypt, Zimbabwe, Cameroon, Kenya, Namibia, Mozambique, Sudan (Africa); Brazil, Haiti, Jamaica, Mexico, the Dominican Republic (Central and South America); Afghanistan, Vietnam, India, China, Malaysia, Thailand (Asia) [8, 9]. In 2017, 45 cases of malaria were imported to Ukraine: 36 (80%) of them were caused by *P. falciparum*. Three patients with tropical malaria died. Besides, 3 cases of ovale malaria, three-day malaria, as well as malaria caused by several pathogens were reported. 28 Ukrainian citizens (62%) and 17 foreigners (38%) suffered. Most cases (60%) happened in the season of possible transmission of the pathogen (April-October 2017). The areas at risk were Kyiv (10 cases (22%)), Kharkiv (9 cases (20%)) and Odesa (8 cases (18%)) regions, as well as in previous years. Most of the infections are imported from Africa: 39 cases (87%) from 13 African countries, the other – from South Asia and America [10].

In 2018, 4 cases of malaria were registered in Ternopil region. Unfortunately, in Ukraine, there are no medications necessary for treatment of malaria.

A case of imported tropical malaria in a pregnant woman has been registered.

The clinical case is presented: A Patient A., 24 years old, a citizen of Nigeria. First pregnancy, 32 weeks. The patient was admitted to the hospital on September 13, 2018 into the First Department of Obstetrics of the Municipal Noncommercial Enterprise “Ternopil City Municipal Hospital No.2” with complaints of high temperature up to 37.4 °C, cold, epigastric and lower abdominal pain.

According to epidemiological anamnesis, she was treated for malaria in her native country in 2017. She arrived to Ukraine on August 31, 2018. The patient stated that she had received a

course of antimalarial drugs before leaving the country; she did not remember their title.

On September 10, 2018, she applied to the Department of Infectious Diseases of Ternopil City Emergency Hospital with complaints of lower abdominal aching pain. She stated that it was associated with eating beans the day before. During the examination by an infectious disease specialist and a surgeon, a body temperature was 36.1 °C, a tonic muscle tension of anterior abdominal wall with muscular guarding, and doubtful symptoms of peritoneal irritation were present that caused assumption of appendicitis.

By agreement with the head obstetrician-gynaecologist of the region, the patient was taken to the Regional Perinatal Centre by an ambulance; she was accompanied by a paramedic and a doctor; the patient stayed in the hospital only for 1 day, because she got better and left the medical facility at her reasonable discretion.

On September 13, 2018, epigastric and lower abdominal pain, cold, high body temperature up to 37.4 °C were present. At the time of examination, the patient's state was of moderate severity. She was anxious. No skin changes were evidenced. Mucous membrane of oropharyngeal cavity was pink. Peripheral lymph nodes were not enlarged. Body temperature was 38.1 °C. Heart rate was rhythmic, tones were muffled. Pulse was 118/min; pulse strength and effort were satisfactory. Blood pressure was 110/70 mm Hg. The lungs: vesicular breathing with a rigid tinge, no wheezing was heard. Respiratory rate was 33/min, SpO₂ – 97.0%. Increased volume of abdomen was evidenced because of pregnancy; it was sensitive to palpation in the region of epigastrium. Pasternatsky symptom was poorly positive, more to the left. Intestinal habits were in norm.

Obstetric examination. The uterine tonus was in norm. The presentation of the foetus was polar; the head was above the pelvic inlet. Amniotic fluid was not broken. Foetal heartbeat was clear, rhythmic; heart rate was 140/min. No oedema was evidenced.

Total blood count (September 14, 2018): erythrocytes 2.32 T/L, haemoglobin 71 g/L platelet count 198 G/L, ESR 65 mm/hour, leucocytosis with left deviation. Biochemical blood tests proved increased levels of creatinine 172.1 µmol/L and urea 14.5 mmol/L. On the ultrasound the liver and spleen were not enlarged; the signs of hydronephrosis on the right, salt diathesis, signs of oligoamnios were present.

In the evening at 8:30 p.m. the patient's state worsened. The patient complained of more severe pain in the epigastrium, general weakness. They were dull, answered to the question unclearly. Body temperature rose up to 38.6 °C. The heart activity was tachyarrhythmic, the tones were suddenly muffled. Pulse was 132/min, pulse strength and effort were poor. Blood pressure was 80/40 mm Hg. The laboratory parameters worsened: erythrocytes 2.15 T/L, haemoglobin 60 g/l, platelet count 190 G/L, ESR 70 mm/hour, vacuolation of neutrophils, significant poikilocytosis. On ultrasound, the size of the liver increased by 3 cm, of the spleen – by 2 cm. Due to the severe state of the patient, at 23:30 p.m. she was transferred to the intensive care unit.

The patient was examined by a duty surgeon, who denied acute surgical pathology. Taking into account epidemiological anamnesis, temperature response, laboratory tests and ultra-

sound examination, an infectious disease specialist was called for consultation. The doctor suspected malaria and referred the patient for some immediate examinations.

After medical consultations of anaesthetists, obstetricians, gynaecologists, infectiologists and surgeons, a preliminary diagnosis was established: first pregnancy, 31-32 weeks. Gestational pyelonephritis. Right hydronephrosis. Salt diathesis. Moderate anaemia in pregnancy. Oligoamnios. Placental dysfunction. Intoxication syndrome of unclear aetiology. Suspicion on malaria.

On September 15, 2018 at 2:00 am the state of the patient was severe. She was conscious, but inadequate, confused mental state, meaningful contact was difficult. The subicteritiousness of sclera was evident. Body temperature was 36.3 °C, pulse 102/min. Blood pressure was 90/60 mm Hg. Heart tones were suddenly weakened. Spontaneous respiration was by an oxygen mask. Auscultatory was weakened in the lower parts. SpO₂ –

96.0%. Obstetric examination: enlarged abdominal because of pregnancy. The uterine tonus was in norm. Fundal height was 31 cm, abdominal circumference – 97 cm. The presentation of the foetus was polar, cephalic. The heartbeat of the foetus was rhythmic, 148/min, muffled. Amniotic fluid was not broken.

The total blood count proved progression of anaemia, the biochemical blood test – hyperbilirubinemia due to direct fraction, moderate activity of aminotransferases, development of nitrogen bases, the coagulogram – decreased prothrombin index up to 85%, increased level of fibrinogen B – (++++), total fibrinogen – 5.7 g/l. Additional abdominal ultrasound confirmed increased sizes of the liver by 3.5 cm, and the spleen by 3 cm. The microscopy of thick-blood film revealed a malarial plasmodium (an immature ring-shaped schizont). *P. falciparum* was found in the blood smear. The level of parasitemia was 240 (++) in 1 ml of blood (Figs. 1-3).

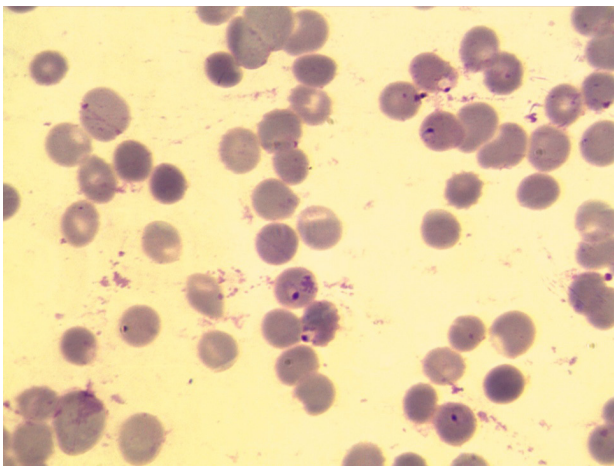


Fig. 1. *P. falciparum*, thick-blood film: numerous ring-shaped parasite forms

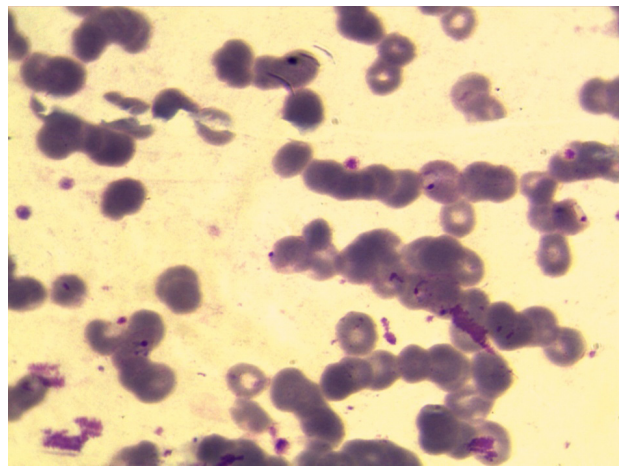


Fig. 2. *P. falciparum*, thin smear: trophozoites of all ages

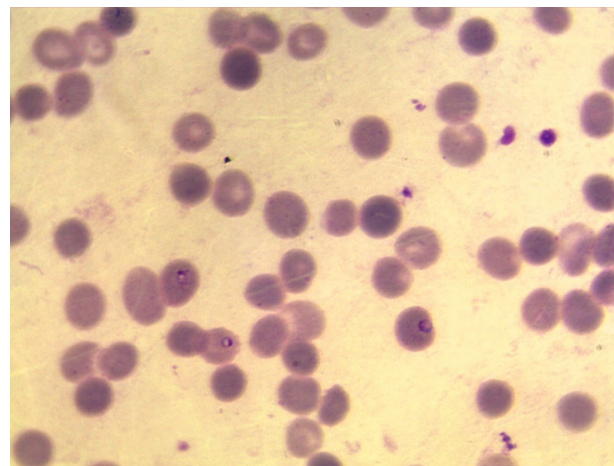


Fig. 3. *P. falciparum*, thick-blood film: numerous ring-shaped parasite forms and trophozoites of all ages

The diagnosis was clarified: tropical malaria (epidemiologically, clinically, and laboratorially). Malaria coma (September 15, 2019), the subcompensation stage. Severe haemolytic anaemia. Acute hepatorenal insufficiency. First pregnancy, 31-32 weeks. Placental dysfunction. Oligoamnios.

Taking into account a high risk of haemorrhagic complications, despite the severe state of the patient, delivery was refused. Dynamic monitoring of the foetal state (cardiotocography

2 times a day) was assigned. In the absence of the necessary etiotropic antimalarial drugs for parenteral administration, Amatem Forte (Artemether 80 mg + Lumefantrine 480 mg) was prescribed: 1 tablet every 8 hours on the 1st day, then 1 tablet twice a day. The patient prudently brought the drug from Nigeria. Additionally, 600 mg of dalacin was administered intravenously twice a day. Taking into account severe anaemia, the patient underwent transfusion of erythrocyte mass B (III) Rh (-),

510 ml. The infusion was conducted according to diuresis and haematocrit parameters in the volume of 3,000 ml in the ratio of crystalloids: colloids 2:1. Some symptomatic agents were prescribed situationally.

The parasitic load was regularly monitored quantitatively twice a day.

On the background of the intensive therapy, the state of the patient improved significantly the next day. As of September 16, 2018 at 9:00 am, her state was moderately severe. Only malaise was present. The patient was active, adequate in communication. Skin was of normal colour. Marginal scleral icterus was evidenced. The tongue was wet, densely covered with white fur. Body temperature was 36.7°C. Respiratory rate was 18/min, pulse – 72/min. Blood pressure was 120/65 mm Hg. Vesicular breathing of the lungs. Increased volume of abdomen was evidenced because of pregnancy. The liver protruded below the costal margin by 1.5-2 cm, the spleen - by 2 cm (instrumentally). The Pasternatsky symptom was negative to both sides. Diuresis was sufficient. Faeces were formed, once a day, with no pathological admixtures.

Laboratorially (September 18, 2018): moderate anaemia, the rates of bilirubin, creatinine, urea were nearly in norm. The microscopy revealed that the level of parasitemia was only 2-3 plasmodia in 1 µl of blood.

Because of the improvement of the patient's state, she was transferred to the Second Department of Obstetrics of Ternopil City Hospital No. 2 for further treatment. The effectiveness of the antimalarial treatment was confirmed by the regression of clinical signs of the disease and parasitoscopically: on September 19, 2018 no malarial plasmodia were found in the native blood. The state of the patient improved significantly, so she was discharged from the hospital, her state was satisfactory. The patient was referred under medical supervision of an infectiologists and obstetrician-gynaecologist. Childbirths took place as expected, on time; a healthy, full-term infant was born.

Tropical malaria is the most severe of all types of this disease. The incubation period is 8-16 days. Then, in some of non-immune persons some prodromes are present which last from several hours to 1-2 days: malaise, weakness, tiredness, body pain, myalgia and arthralgia, headache. In most patients, tropical malaria starts acutely, without any premonitory symptoms and with a body temperature up to 38-39°C. The presence of several main genii of *P. falciparum* in the infected organism with different end-time cycles of erythrocytic schizogony is clinically manifested in the irregular periodicity of fever paroxysms or their absence. Usually, the attack begins with a fever that lasts from 30 minutes to 1 hour. In this period, the skin is pale, cold, often with dermal reflex. Algid coincide in time with a rise of body temperature up to 38-39 °C. High body temperature, the second phase of paroxysm, takes place after algid. Patients experience a feeling of warmth, sometimes heat. The skin is hot, the face is hyperaemic. This phase lasts about 12 hours, after it the patient sweats profusely. In cases of cyclic infectious process, the body temperature falls to normal and subnormal rates and in 1-2 hours rises again. Sometimes tropical malaria begins with nausea, vomiting, diarrhoea. Catarrhal symptoms may take place in upper respiratory tract: cough, runny nose, sore throat. At the later stage a herpetic rash on the lips and nose alae is present. At the acute stage hyperaemia of conjunctiva is significant, which in cases of the severe degree of the disease may be accompanied by petechial or subconjunctival haemorrhages. In the midst of malaria, algid is weaker than in the early period of the disease, its duration is only 15-30 minutes. Fever lasts around the clock,

epirexyzes are rarely registered. In cases of mild course of the disease, the body temperature rises up to 38.5°C, the duration of fever is 3-4 days; in cases of a moderate disease, respectively, up to 39-39.5°C and 6-7 days. In cases of severe malaria the rise of body temperature up to 40°C and more with the duration for 8 days or more is typical. In cases of tropical malaria, some paroxysms (in fact, the layers of several paroxysms) last 30-40 hours. The wrong type of temperature curve predominates; the remitting one – rarely, occasionally the intermittent and constant types.

The enlargement of liver and spleen is usually evidenced on the 3rd day of the illness, however, splenomegaly can be revealed only percussively, its clear palpation is possible only on the 5-6th day. On abdominal ultrasound, the increased sizes of liver and spleen are evident already on the 2nd-3rd day after the onset of the clinical manifestations of tropical malaria. Pigment metabolism disorders are present only in the patients with severe and moderate (more rarely) degree of the disease. More than three-time increase of serum aminotransferase activity is regarded as an indicator for unfavourable prognosis. Metabolic disorders comprise changes in haemostasis and hypoglycaemia.

Cardiac and vascular disorders are functional: tachycardia, muffled heart tones, hypotension. Occasionally, a transient systolic murmur at the top of heart is heard. In cases of severe degree of the disease the changes in the ECG are present in the form of deformation of the ending part of ventricular complex: flattening and inverse configuration of the T wave, reducing of ST segment. At the same time, the voltage of the waves R in the standard leads reduces. In the patients with cerebral form, the changes of the P wave are of the *P-pulmonale* type. Often, disorders of the central nervous system associated with high fever and intoxication are present. Patients complain of headache, often with vomiting, meningitis, convulsions, drowsiness, and sometimes confused mental state. Haemolytic anaemia and leukopenia are typical signs of moderate to severe malaria, as well as eosinopenia and neutropenia, relative lymphocytosis in the leukocyte formula. In cases of a severe degree of the disease, neutrophilic leukocytosis is often evidenced; ESR is significantly increased at all times. Thrombocytopenia and transient fever albuminuria are typical [1-5].

Relapses of tropical malaria are associated with either inadequate etiotropic treatment, or resistance of *P. falciparum* to chemotherapeutic agents used. This malaria with a favourable outcome lasts no more than 2 weeks. In the absence of etiotropic therapy, relapses take place in 7-10 days.

Pregnancy is an established risk factor for tropical malaria. This is associated with a higher morbidity of pregnant women, severe course, risk to the health and life of a child, limited therapeutic arsenal. Tropical malaria in children under 5 years of age is a potentially lethal disease; in cases of it there are no significant clinical symptoms – malaria paroxysm. At the same time, almost always seizures, vomiting, diarrhoea, abdominal pain with a rapid worsening of the child's state are evidenced. Seizures and other cerebral symptoms do not necessarily point to development of cerebral malaria. They are often the symptoms of neurotoxicosis. Parasitemia in small children is usually significant: *P. falciparum* can affect up to 20% of erythrocytes. The disease can rapidly have a severe course and fatal outcome in a child.

The diagnosis is confirmed by means of microscopy of a thick-blood film and blood smear with colouring by the Romanovsky-Gimz method. The microscopy defines the massivity of invasion (the number of parasites in 1 µl of blood), that is easier to reveal

in a thick-blood film, as well as the type of plasmodium. In the case of a negative result of microscopic examination, the diagnosis of malaria cannot be immediately discarded, because the study should be conducted many times. The level of parasitemia allows defining disease severity, effectiveness of treatment, as well as making prognosis. The WHO recommends the level of parasitemia to be determined by the results of a survey of 100 fields of vision of a thick-blood film [1,11].

The resistance of *P. falciparum* to chloroquine is widespread in most of the world (except for Central America to the west of the Panama Canal, Haiti and the Dominican Republic, as well as some parts of the Middle East).

The WHO and the CDC (Centres for Disease Control and Prevention) recommend first of all a combination of artemisinin three-day therapy together with artemeter/lumefantrine [12]. It is evidenced that in the endemic regions, such a course is safe and quite effective in cases of uncomplicated malaria, as well as in the malaria nonimmune travellers [13]. However, in many countries, these drugs are not licensed or not available.

The American Centre for Disease Control and Prevention (CDC) recommends taking artemeter/lumefantrine or atovaquone/proguanil for 3 days, quinine in combination with doxycycline, tetracycline or clindamycin may be used as an alternative, although the advantage is offered to doxycycline or tetracycline. This is primarily due to the fact that more information about these drugs is available. However, doxycycline or tetracycline is contraindicated in pregnant women and children under 8 years of age. Mefloquine is one more alternative medication. The treatment with mefloquine should be prescribed only if other derivatives are unavailable due to the increased frequency of adverse reactions and concerns about possible psycho-neurological complications. The course of treatment with quinine lasts 7 days for invasions acquired in South-East Asia, and within 3 days for other regions [4].

Conclusions. Thus, in the presence of an appropriate epidemiological anamnesis, the patients with fever of unknown genesis should first of all be examined for malaria, the most socially significant tropical disease. It is necessary to define the type of malarial plasmodium by repeated blood parasitology by a thick-blood film and blood smear coloured by Gimza-Romanovsky method. Doctors' vigilance against malaria allows preventing complicated forms and late relapses of this malignant invasion. The countries free of malaria can also face this problem and therefore they should be ready to diagnose and treat this disease effectively. The infectious diseases hospitals of Ukraine should be supplied with antimalarial drugs.

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SUMMARY

IMPORTED TROPICAL MALARIA (CASE REPORT)

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Malaria is a parasitic disease. It is one of blood infections caused by malaria plasmodia. The disease is transmitted to a human by a bite of a female mosquito of *Anopheles* genus. Local malaria transmission in Ukraine has not been registered since 1956, however, every year some imported cases occur. In 2017, 45 cases of malaria were imported to Ukraine: 80% of them were caused by *P. falciparum*.

The aim of the research is to present a case of imported tropical malaria in a pregnant woman with the development of malaria coma. An unusual course of the illness made diagnoses difficult due to partial immunity of the patient caused by multiple previous invasions of malaria plasmodia. The diagnosis was confirmed by blood microscopy. A literature on epidemiology, clinical findings and current tropical malaria course has been scanned as well.

In the presence of an appropriate epidemiological anamnesis, the patients with fever of unknown genesis should first of all be examined for malaria, the most socially significant tropical disease. It is necessary to define the type of malarial plasmodium by repeated blood parasitology by a thick-blood film and blood smear coloured by Gimza-Romanovsky method. Doctors' vigilance against malaria allows preventing complicated forms

and late relapses of this malignant invasion. The countries free of malaria can also face this problem and therefore they should be ready to diagnose and treat this disease effectively. The infectious diseases hospitals of Ukraine should be supplied with antimalarial drugs.

Keywords: tropical malaria, malaria coma, pregnancy.

РЕЗЮМЕ

ЗАВОЗНАЯ ТРОПИЧЕСКАЯ МАЛЯРИЯ (СЛУЧАЙ ИЗ ПРАКТИКИ)

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Малярия является паразитарной болезнью крови, вызванной малярийным плазмодием. Болезнь передается человеку от укуса самки комара рода *Anopheles*. Местная передача малярии в Украине не регистрируется с 1956 г., однако каждый год происходит несколько завозных случаев. Так, в 2017 г. в Украине зарегистрировано 45 случаев завозной малярии, 80% из них вызваны *P. falciparum*.

Цель исследования – описание случая завозной тропической малярии у беременной женщины с развитием малярийной комы.

Диагностику затрудняло нетипичное течение болезни, вызванное частичной иммунностью пациентки, обусловленной неоднократными предварительными инвазиями малярийного плазмодия. Диагноз подтвержден микроскопией крови.

Представлен обзор литературы по эпидемиологии, клинике и современному течению тропической малярии. При наличии соответствующего эпидемиологического анамнеза больные лихорадкой неясного генеза, прежде всего, должны обследоваться на предмет наиболее социально значимой тропической болезни – малярии. Обязательным является установление вида малярийного плазмодия путем многократной паразитоскопии крови методами толстой капли и тонкого мазка, окрашенными по Гимза-Романовскому. Соответствующая настороженность врачей относительно

малярии позволит избежать осложненных форм и отдаленных рецидивов этой опасной инвазии. Страны, свободные от малярии, могут столкнуться с этой проблемой и поэтому должны быть готовы к диагностике и лечению этой болезни. В инфекционных стационарах Украины целесообразно иметь запас противомаларийных препаратов.

რეზიუმე

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კვლევის მიზანს წარმოადგენდა მალარიის კომის მქონე ფეხმძიმე ქალში შემოტანილი ტროპიკული მალარიის შემთხვევის აღწერა. დიაგნოსტიკას ართულებდა პაციენტის ნაწილობრივი იმუნურობით გამოწვეული დაავადების არატიპური მიმდინარეობა, რისი მიზეზიც იყო მალარიის პლაზმოდით არაერთჯერადი წინასწარი ინვაზია. დიაგნოზი დადასტურდა სისხლის მიკროსკოპირებით.

ლიტერატურის მიმოხილვაში წარმოდგენილია ტროპიკული მალარიის ეპიდემიოლოგია, კლინიკა და მიმდინარეობა. უცნობი ეტიოლოგიის ცხელების მქონე პაციენტები, რომელთაც აქვთ შესაბამისი ეპიდემიოლოგიური ანამნეზი, გამოკვლეული უნდა იყვნენ სოციალური მნიშვნელობის ტროპიკულ დაავადებაზე - მალარიაზე. აუცილებელია მალარიის პლაზმოდით სისხლის დადგენა, რაც ხდება სისხლის მსხვილი წვეთისა და თხელი ნაცხის მეთოდებით მრავალჯერადი პარაზიტოსკოპიით, გიმზა-რომანოვსკის წესით შედეგით. მალარიასთან დაკავშირებით ექიმთა სიფრთხილე თავიდან აგვაცილებს ამ საშიში ინვაზიის გართულებულ ფორმებსა და შორეულ რეციდივებს. ასეთი პრობლემის წინაშე შესაძლოა დადგნენ მალარიისგან თავისუფალი ქვეყნები და ამიტომ, ისინი მზად უნდა იყვნენ ამ დაავადების სადიაგნოსტიკოდ და სამკურნალოდ. მიზანშეწონილია, რომ უკრაინის ინფექციურ სტაციონარებში იყოს მალარიის საწინააღმდეგო პრეპარატების მარაგი.

КОМПЛЕКСНОЕ ИЗУЧЕНИЕ БИОЛОГИЧЕСКОГО ДЕЙСТВИЯ ИНГАЛЯЦИЙ РАДОНОВОЙ ВОДОЙ ЦХАЛТУБО

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В настоящее время особенно актуальной [6,10] является борьба с антропологическими последствиями воздействия электромагнитного (ЭМП) загрязнения окружающей среды, которое в форме коммуникационных и сетевых частот особенно доминирует в жилых и рабоче-производственных

помещениях. Согласно современным представлениям [2,7], длительное нахождение в этом поле вызывает значительные изменения в крови и моче. Среди мультиорганных эффектов, связанных с воздействием ЭМП, особое место занимают нейротропное и психотропное воздействия (минималь-

ная дисфункция ЦНС), поскольку нервная система, наряду с гемopoетической и эндокринной системами особенно чувствительна к воздействию ЭМП. Указанные изменения требуют строгой профилактической коррекции. Пути лечебно-профилактической коррекции, несмотря на незначительную выраженность, в настоящее время активно исследуются как в биологическом, так и клиническом аспектах [4]. Исходя из изложенного, особую актуальность приобретают лечебно-реабилитационные мероприятия немедикаментозного характера, в частности бальнеотерапия, которая является существенной альтернативой побочных эффектов лекарственного воздействия [9].

Хорошо известны и пользуются широкой популярностью радоносодержащие воды Цхалтубо, которым посвящено множество исследований медико-биологического характера. Процедуры общего воздействия на организм пациентов, целиком погруженных в ванны, достаточно хорошо исследованы [3]. Результаты локального применения радоновых вод (различные формы промываний, полоскание полости рта, ингаляции) по сей день недостаточно изучены. Одной из немногих работ является статья Nikolaishvili M. et al [8] о положительной роли локального воздействия на ротовую полость (оральное полоскание) радоновых вод Цхалтубо при периодонтитах, возможности блокады их первичных форм, об оптимизации минерализации периодонта и нормализации его кровоснабжения.

Локальное воздействие вдыхаемой в открытом пространстве воздушной фазы радоносодержащей воды Цхалтубо практически не исследованы и требуют дальнейших клинико-лабораторных наблюдений. Актуальность подобных исследований особенно высока [1], так как в открытом пространстве, при температуре протекающей воды 36-37°C, упругость насыщения ее водяного пара составляет почти 32,8 мл. ртутного столба, что достаточно для успешной альфатерапии [5]. Однако, такие данные в научной литературе не обнаружены.

Целью исследования явилось определить характер действия ингаляции радоновой водой Цхалтубо на сомато-вегетативные жалобы пациентов, более десяти лет находившихся в зоне воздействия электромагнитного поля коммуникационной и сетевой частот, а также композицию биогенных аминов и свободных аминокислот плазмы крови до и после альфатерапии.

Материал и методы. Наблюдаемый контингент состоял из 25 женщин (средний возраст 32 года), постоянно находившихся (служебные обстоятельства, семейные привычки) в течении более 10 лет в зоне ЭМП коммуникационной и сетевой частот. Несмотря на отсутствие органических нарушений (данные анамнеза) в течение последних 2-3 лет пациенты жаловались на постоянное присутствие дифференцированных форм повышенной раздражительности, рекуррентные депрессивные эпизоды легкой степени, часто переходящие в необоснованную усталость, локальные и общие головные боли, сонливость с нарушениями режима сна. Так как эта симптоматика носила минимально выраженный характер, была непостоянна и не вызывала озабоченности, указанные расстройства не соответствовали группе F-45 (МКБ-10) и, согласно современным представлениям, являлись следствием воздействия ЭМП коммуникационной частоты [5,7].

В сидячем положении, на расстоянии 50,0 см от края бассейна и 100,0 см над протекающей водой, проводилась 10-минутная ингаляция воздушной фазы радоносодержащего (37,0 Вq/m³) источника. Учитывая, что в открытой воздуш-

ной системе смешивание воздушной фазы в легких длится около 3 минут (индекс эффективности 62-98%, упругость насыщения 32,8 мн.ртут.столба) 10-минутная ингаляция вполне достаточна для альфатерапии при количестве дыхательных движений 16-18 в минуту и дыхательном объеме 500,0 мл. Общая радиационная доза не определялась, она была значительно ниже допустимых предельно допустимых концентраций. Проведено 10 процедур.

Количественный анализ свободных аминокислот плазмы крови проведен на автоматическом аминокислотном анализаторе ААА-339 (Чехословакия), бета-эндорфинов - радиоиммунным методом (набор Medicor&inc.) катехоламинов - ELIZA Kit. Данные представлены в средних стандартных отклонениях (SAM) и обработаны в программе SPSS v.16.0.

Результаты и их обсуждение. Согласно проведенным наблюдениям, ингаляция радоновой водой Цхалтубо положительно воздействует на сомато-вегетативные недомогания исследуемого контингента. Уже после пятой ингаляционной процедуры жалобы на повышенную раздражительность, а также локальные и общие головные боли исчезали. Частота рекуррентных депрессивных эпизодов после восьмой ингаляционной процедуры немного снизилась, а после десятой - полностью пропала. На десятой ингаляции контингент не жаловался на нарушения режима сна, полностью исчезло ощущение сонливости, усталости. Все обследуемые проявляли положительную эмоциональную активность.

Клинико-психологический анализ наступивших положительных изменений, динамики и глубины их развития не входили в структуру проводимых исследований, поэтому приводятся только данные, связанные с содержанием и композицией биогенных аминов и свободных аминокислот плазмы крови до и после проведенных ингаляционных процедур. Известно, что указанные компоненты плазмы прямо или опосредованно регулируют все основные психо-нервные процессы: возбуждение и торможение, бодрость и сонливость, агрессию и тревогу, поведение, память.

Режим питания у обследуемых был одинаковым. Характер энергетических затрат и потребностей сбалансированного питания по А.А. Покровскому (незаменимые, заменимые аминокислоты, углеводы, жиры) не исследован. Классификация свободных аминокислот плазмы проведена по М.Ф. Мережинскому и Л.М. Черкасовой [1], согласно свойствам радикала R, определяющего их химическую природу.

Согласно приведённым данным ингаляция радоновой водой влияла на уровень отдельных биогенных аминов плазмы по-разному. После десятой ингаляции содержания дофамина в плазме пациентов увеличилось до 79,9±36 пг/мл (p<0,01), норадреналина - достоверно уменьшилось - 70,2±3,1 пг/мл, (p<0,001). Уменьшились также показатели уровня серотонина - 64,2±2,9 нг/мл, (p<0,001) и эндорфинов - 9,4±1,2 пмол/л, (p<0,01) плазмы. Они до проведенных процедур были достаточно высокими - 86,9±3,2 нг/мл и 12,7±1,1 пмол/, соответственно (таблица 1).

Высокий уровень бета-эндорфинов в плазме крови пациентов до проведенных процедур (12,7±1,1 пмоль/л), по всей вероятности, является следствием компенсаторного реагирования на пониженное содержание дофамина (54,8±2,6 пг/мл) у пациентов, находившихся длительное время в зоне ЭМП коммуникационной и сетевой частот. После альфатерапии (десятая ингаляция) содержание дофамина значительно возросло (79,9±3,6 пг/мл, p<0,001), а бета-эндорфинов соответственно снизилось (9,4±1,2 пмоль/л). Указанные биогенные амины принимают значимое участие в вопросах

Таблица 1. Усредненные показатели содержания биогенных аминов в плазме крови пациентов до и после ингаляции

До ингаляции				После ингаляции			
Дофамин	Норадреналин	Серотонин	β-эндорфины Пикомоль/л	Дофамин	Норадреналин	Серотонин	β-эндорфины Пикомоль/л
54,8±2,6	92,4±2,9	86,9±3,2	12,7±1,1	79,9±3,6	70,2±3,1	64,2±2,9	9,4±1,2

Таблица 2. Усредненные показатели содержания свободных аминокислот в плазме крови пациентов до и после ингаляции (мг/100 мл плазмы, МКСА)

Аминокислоты	До ингаляции	После ингаляции	Аминокислоты	До ингаляции	После ингаляции
<u>Гидрофобные – с неполярным радикалом</u>					
Аланин	6,10 ± 0,08	3,06 ± 0,02	Пролин	1,89 ± 0,02	2,01 ± 0,03
Валин	2,45 ± 0,01	3,89 ± 0,02	Фенилаланин	2,01 ± 0,02	3,40 ± 0,02
Лейцин	2,88 ± 0,02	3,09 ± 0,01	Триптофан	1,20 ± 0,01	2,19 ± 0,02
Изолейцин	1,98 ± 0,01	2,10 ± 0,01	метионин	0,49 ± 0,01	1,06 ± 0,01
<u>С полярным (незаряженным) радикалом</u>					
Глицин	4,08 ± 0,03	1,79 ± 0,02	Амид глутаминовой кислоты (глутамин)	0,56 ± 0,01	1,08 ± 0,01
Серин	1,09 ± 0,02	1,20 ± 0,02	Амид аспарагиновой кислоты (аспарагин)	0,89 ± 0,01	1,48 ± 0,01
Тирозин	0,88 ± 0,01	1,92 ± 0,01			
Треонин	1,40 ± 0,01	2,18 ± 0,01			
Цистеин	1,08 ± 0,01	1,36 ± 0,01			
<u>Кислые – с отрицательно заряженным радикалом</u>					
Аспарагиновая кислота	0,08 ± 0,01	0,24 ± 0,02	Глутаминовая кислота	0,78 ± 0,01	1,27 ± 0,02
<u>Основные – с положительно заряженным радикалом</u>					
Лизин аргинин	2,14 ± 0,02 0,82 ± 0,01	4,86 ± 0,03 1,66 ± 0,01	Гистидин	2,92 ± 0,02	1,04 ± 0,02

регуляции уровня ряда гормонов, в частности тестостерона и пролактина, поэтому исследование влияния ингаляции радоновой водой Цхалтубо на процессы гликолиза клинически перспективны.

Согласно полученным данным, ингаляция радоновой водой Цхалтубо значительно повлияла на композицию и уровень свободных аминокислот плазмы крови пациентов. В зависимости от свойств радикала, определяющего химическую природу аминокислот, характер воздействия радонсодержащей водой Цхалтубо оказался неодинаковым (таблица 2).

После проведенной альфатерапии в плазме крови значительно снизился уровень аланина. Являясь аминокислотой с неполярным радикалом, его содержание до проведенной ингаляции достигло 6,10±0,08 мг/100 мл, а после ингаляции не превышало 3,06±0,02 мг/100 мл (p<0,001). Как заменимая моноаминомонокарбоновая аминокислота (ее суточная потребность достаточно велика), аланин и его производные составляют около 60-65% содержащихся в белках аминокислот и легко синтезируется в организме. Конкуренция в механизме всасывания аланина, как продукта гидролиза не обнаружена.

Из аминокислот с неполярным радикалом ингаляция радоновой водой не изменила содержание в плазме крови моноаминомонокарбоновой кислоты с гидрофобным радикалом – изолейцина. Его уровень как до, так и после проведенных процедур оказался достоверно одинаковым, 1,98±0,01 мг/100 мл и 2,10±0,01 мг/100 мл, соответственно, p>0,05. Альфатерапия достоверно не изменила также содержание аминокислоты пролина. Незаменимая аминокислота не содержит свободной аминогруппы и обладает неполяр-

ным радикалом: до - 1,89±0,02 мг/100 мл и после - 2,01±0,03 мг/100 мл проведенной альфатерапии его содержание практически одинаково (p>0,05). Уровень остальных аминокислот с неполярным радикалом достоверно увеличился. Содержание валина и лейцина, как незаменимых аминокислот увеличилось до 3,89±0,02 и 3,09±0,01 мг/100 мл. (p<0,01). Их суточная потребность для взрослого организма особенно велика. Фенилаланин относится к группе незаменимых аминокислот и его оптимальный уровень в организме также связан с поступлением пищевых продуктов. Его потребность очень велика и может покрываться также эндогенным путем в результате протеолиза некоторых белков. Однако, этот процесс минимален. После ингаляционных сеансов его уровень в плазме крови значительно возрос (3,40±0,02 мг/100 мл, p<0,01). В этом отношении необходимо отметить значение превращения фенилаланина в тирозин. Так называемое гидроксирование, которое является ферментативным процессом, катализируемым двумя различными ферментами, может осуществляться только в аэробных условиях, т.е. при поступлении достаточного количества артериальной крови. После проведенных ингаляций в плазме крови пациентов значительно увеличился уровень незаменимой гетероциклической аминокислоты-триптофана (до 1,20±0,01, после 2,19±0,02 мг/100 мл, p<0,01). В обмене триптофана наиболее важное значение приобретает биосинтез серотонина и образование никотиновой кислоты. Серотонин играет важнейшую роль в высшей нервной деятельности. Другой путь превращения триптофана (образование никотиновой кислоты) обычно сбалансирован с экзогенным

поступлением никотиновой кислоты из пищевых продуктов, в результате чего эндогенное образование никотиновой кислоты из триптофана резко меняется. Указанный путь схож, с одной стороны, с энергетическим метаболизмом, а с другой - окислительно-восстановительными процессами. При декарбоксилации триптофан превращается в физиологически активное соединение – триптамин, вызывающий повышение кровяного давления.

Кроме процессов дезаминирования и перезаминирования осуществляются также особые превращения, свойственные только данным аминокислотам. В этом аспекте значимую роль играет метионин, как липотропный фактор, участвующий в процессах переметилирования и синтеза холина. Однако, превращения метионина возможны только после его перехода в активное состояние, т.е. получения добавочных количеств сульфатных радикалов, участвующих в детоксикационных реакциях. Активирование осуществляется специальными ферментными системами печени и АТФ. После проведенных ингаляций в плазме крови пациентов особенно увеличился уровень метионина ($p < 0,001$) – серосодержащей аминокислоты с гидрофобным радикалом, предшественником – аденозилметионина, универсального донора процессов переметилирования, $0,49 \pm 0,01$ и $1,06 \pm 0,01$ мг/100 мл, соответственно. Метионин как исключительно значимая аминокислота является источником добавочного количества сульфатных радикалов, участвующих в детоксикационных процессах. В условиях снижения интенсивности реакций переметилирования, обменные превращения метионина смещаются в сторону образования метилмеркаптана.

После ингаляции радоном в группе аминокислот с полярным (незаряженным) радикалом значительно снизился уровень простейшей по строению аминокислоты – глицина. Являясь предшественником синтеза ряда биологически активных соединений (порфирины, пурины, глутатион, гликолевая кислота, гиппуровая кислота, креатинин) эта моноаминомонокарбоновая аминокислота отличается весьма внушительной суточной потребностью. До ингаляции его уровень в плазме крови пациентов достигал $4,08 \pm 0,03$ мг/100 мл, а после проведенных процедур не превышал $1,79 \pm 0,02$ мг/100 мл. различие достоверно ($p < 0,001$). Не изменилось только содержание α -амино- β -оксипропионовой кислоты-серина. Являясь моноаминомонокарбоновой кислотой с полярным (незаряженным) радикалом, до альфатерапии и после альфатерапии его уровень в плазме крови пациентов достоверно не изменился - $1,09 \pm 0,02$ и $1,20 \pm 0,03$ мг/100 мл, соответственно, $p > 0,05$. Его наиболее значимым превращением в организме является пировиноградная кислота, играющая важнейшую роль в процессах обмена веществ. Присутствуя в большинстве тканей организма она служит главным переходным веществом при биосинтезе белков из углеводов и наоборот.

Согласно полученным данным, проведенные ингаляции, значительно увеличили уровень отдельных аминокислот с полярным (незаряженным) радикалом. До начала процедур, уровень тирозина в плазме крови пациентов не превышал $0,88 \pm 0,01$ мг/100 мл, а после проведенных процедур увеличился до $1,92 \pm 0,01$ мг/100 мл. Различие достоверно ($p < 0,01$). Ароматическая гомоциклическая аминокислота является значимым предшественником синтеза гормонов щитовидной железы, а также процессов функционирования мозгового слоя надпочечников. Метаболизм тирозина связан с разрывом бензольного кольца и образованием фумариновой и ацетоуксусной кислот. Для гидроксирования тирозина в мозговом слое надпочечников требуется участие

фенолоксидазы. Производными тирозина являются азотсодержащие основные гормоны щитовидной железы: тироксин и трийодтироксин, а также важнейшие гормоны мозгового слоя надпочечников-норадреналин, адреналин. Существует мнение о связи между расстройствами высших функций нервной системы и нарушениями метаболизма тирозина. Важное значение имеет образование тирозина из фенилаланина. Уровень треонина также значительно увеличился. До ингаляции $1,40 \pm 0,01$ мг/100 мл после ингаляции $2,18 \pm 0,01$ мг/100 мл. Моноаминокарбоновая кислота обычно ($p < 0,01$) распадается с образованием глицина и уксусной кислоты. Вместе с этим треонин может превращаться в α – кетокислоту.

Уровень серосодержащей аминокислоты цистеина также достоверно увеличился. До проведенных ингаляций он не превышал $1,08 \pm 0,01$ мг/100 мл, а после десятого сеанса – $1,36 \pm 0,01$ мг/100 мл, $p < 0,01$. Содержание амидов глутаминовой и аспарагиновой кислот также достоверно возросло, $p < 0,01$: $0,56 \pm 0,01$ и $1,08 \pm 0,01$ мг/100 мл (глутамин) и $0,89 \pm 0,01$ и $1,48 \pm 0,01$ мг/100 мл (аспарагин).

В группе кислых, с отрицательно заряженным радикалом аминокислот, достоверно увеличилось ($p < 0,01$) уровни аспарагиновой и глутаминовой кислот. Аспарагиновая кислота (до ингаляции $0,08 \pm 0,01$, после ингаляции $0,24 \pm 0,02$ мг/100 мл) широко участвует в метаболических процессах. Она предшествует синтезу пиримидинов и пуринов. Превращается в щавелеуксусную кислоту. Глутаминовая кислота (до ингаляции $0,78 \pm 0,01$, после проведенных ингаляций $1,27 \pm 0,02$ мг/100 мл) является предшественником глутатиона. В организме существуют высокоактивные соединения, катализирующие превращение глутаминовой кислоты в α -кетоглутаровую. В группе основных аминокислот с положительно заряженным радикалом, значительно уменьшился уровень гистидина. До проведенных процедур он достигал $2,92 \pm 0,02$ мг/100 мл, а после окончания ингаляций не превышал $1,04 \pm 0,02$ мг/100 мл ($p < 0,001$). Имидазольное кольцо этой гетероциклической аминокислоты обладает способностью протонирования и депротонирования при физиологических значениях pH. Значимый медиатор нервной системы – гистамин образуется при декарбоксилации гистидина. Ингаляция радоном также способствовала значительному увеличению уровня лизина (до процедур $2,14 \pm 0,02$ мг/100 мл, после процедур $3,86 \pm 0,03$ мг/100 мл) и аргинина (до процедур $0,82 \pm 0,01$ мг/100 мл, после процедур $1,66 \pm 0,01$ мг/100 мл). Аргинин является значимым компонентом цикла образования мочевины и широко участвует в метаболических процессах. Основные белки (хроматин, рибосомальные белки) содержат в большом количестве лизин.

Таким образом, ингаляция радонсодержащей водой, значительно повлияла на конфигурацию аминокислотного состава и уровень биогенных аминов плазмы обследованных. После десятой процедуры содержание дофамина в плазме крови достоверно возросло, а норадреналина, серотонина и β -эндорфинов достоверно снизилось. Радонсодержащая ингаляция вызвала увеличение уровней незаменимых аминокислот плазмы. Вместе с этим, уровни гидрофобного, с неполярным радикалом – аланина, с полярным (незаряженным) радикалом – глицина и основного, с положительно заряженным радикалом – гистидина достоверно снизились. Не изменилось только содержание изолейцина (гидрофобная аминокислота с неполярным радикалом) и серина - аминокислота с полярным (незаряженным) радикалом.

Увеличение в крови глутамата и аспартата с одновременным снижением глицина и аланина видимо значительно

воздействовало на отношение возбуждающих/тормозящих аминокислот и выразилось в форме положительного клинического резонанса.

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SUMMARY

COMPLEX STUDY OF BIOLOGICAL EFFECT OF TSKHALTUBO RADON WATER INHALATION

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The effect of EMF of high frequencies (mobile phones and computers) and the action of radon therapeutic procedures (phenomenological and influencing therapeutic factors) is not still generally known. In addition, we are constantly under the influence of different EMF frequencies, the study of which also deserves attention.

The aim of the work is detailed analysis of alpha radiation Tskhaltubo water effect. The subject of the study was 25 patients. The group took the inhalation procedure of air radon baths which was 36-37°C, and radon concentration 37.0 Bq/m³. The conducted quantities and qualitative analyses show, that radon inhalation takes an active part in metabolism of biological active components: catecholamine's, amines and free amino acids. Biochemical experiments showed the normalization tendency of composition of these necessary blood-components after 10th day radon-therapeutic inhalation procedure.

Keywords: Tskhaltubo radon water, inhalation, biological active blood-plasma components.

РЕЗЮМЕ

КОМПЛЕКСНОЕ ИЗУЧЕНИЕ БИОЛОГИЧЕСКОГО ДЕЙСТВИЯ ИНГАЛЯЦИЙ РАДОНОВОЙ ВОДОЙ ЦХАЛТУБО

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Определен характер действия ингаляции радоновой водой Цхалтубо на сомато-вегетативные жалобы 25 пациентов (женщины, средний возраст 32 г) более десяти лет находившихся в поле ЭМП коммуникационной и сетевой частот, а также композицию биогенных аминов, и свободных аминокислот плазмы крови до и после альфатерапии. Ингаляция радоносодержащей водой положительно повлияла как на обще-соматическое состояние пациентов так и оптимизировала конфигурацию аминокислотного состава и уровни катехоламинов плазмы крови.

После десятой процедуры (по 10 минут каждая) сомато-вегетативные жалобы полностью нивелировались и потеряли актуальность. Вопросы ингаляции, как формы локального воздействия вдыхаемой в открытом пространстве воздушной фазы радонсодержащей воды Цхалтубо практически не исследованы и требуют дальнейшего клинико-лабораторного изучения. Антропологические последствия воздействия ЭМП окружающей среды, невзирая на их минимальность, требуют обязательную профилактическую коррекцию.

რეზიუმე

წყალტუბოს წყლის ინგალაციის სამკურნალო თვისებების კომპლექსური შესწავლა

მ. ნიკოლაიშვილი, დ. ზურაბაშვილი, თ. მუსელიანი, გ. ჯიკია, გ. ფარულავა

ნ. ბერიტაშვილის ექსპერიმენტული ბიომედიცინის ცენტრი; ი. ჯავახიშვილის სახ. თბილისის სახელმწიფო უნივერსიტეტი; საქართველოს სახელმწიფო ფიზიკური აღზრდის და სპორტის ინსტიტუტი, საქართველო

ეკოლოგიური გარემო, სულ უფრო რთული ხდება, რადგან ბევრი საწარმოო ნარჩენები და სხვა ფაქტორები, როგორცაა ჩვენ შემთხვევაში ელექტრო მაგ-

ნიტური ველები გავლენას ახდენენ ადამიანის ორგანიზმზე და მის ჯანმრთელობაზე. ჩვენი ცდებიდან გამომდინარე, მაღალი სისწილის EMF-ის გავლენის ქვეშ მყოფ პაციენტებში (25 პაციენტი, საშუალო ასაკი 32 წ), რომლებიც ხანგრძლივად იმყოფებოდნენ კომუნიკაციური და ქსელის სისწილის ქვეშ შეისწავლებოდა წყალტუბოს წყლიდან გამოყოფილი რადონის ინგალაცია - (ალფა-თერაპია), პაციენტებში განვითარებულ ვეგეტატიურ ჩივილებზე. კერძოდ, სისხლში განისაზღვრებოდა ბიოგენური ამინები და თავისუფალი ამინომჟავები რადონო თერაპიამდე და თერაპიის შემდეგ.

ცდებიდან ირკვევა, რომ ალფა თერაპია დადებითად მოქმედებს არამარტო პაციენტების საერთოსმატურ მდგომარეობაზე, არამედ გავლენას ახდენს თავისუფალი ამინომჟავების კონფიგურაციის ოპტიმიზაციაზე. 10 პროცენტის შემდეგ აღვილი აქვს სომატოვეგეტატიური ჩივილების ნიველირებას. მიღებული ცდებიდან შეგვიძლია დავასკვნათ, რომ ალფა-რადონო თერაპია დადებითად მოქმედებს ელექტრომაგნიტური ველით განვითარებულ სომატოვეგეტატიურ ჩივილებზე და ათდღიანი ინგალაციის შემდეგ აღვილი აქვს ამ ჩივილების ნიველირებას.

СТРУКТУРНЫЕ ФОРМЫ ФОЛЛИКУЛ-АССОЦИИРОВАННОГО ЭПИТЕЛИЯ ПЕЙЕРОВЫХ БЛЯШЕК ТОНКОЙ КИШКИ БЕЛЫХ КРЫС

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В настоящее время фолликул-ассоциированным эпителием называют поляризованный монослой разного типа энтероцитов, покрывающих люминальную или апикальную поверхность купола лимфоидных узелков пейеровых бляшек, которые еще именуют купольными эпителиальными клетками [4,7,11,18,20]. Согласно общепринятой концепции, ведущая роль в инициальных иммунных реакциях кишечника принадлежит особым клеткам, которые находятся среди данных энтероцитов, составляя примерно 10% от их общего числа. Они получили широкую известность под названием М-клеток. Индекс «М» трактуется по-разному; в одних случаях он обозначает их в качестве клеток медиаторов, т.е. посредников, а другие авторы связывают его с микроскладчатой формой их апикальной поверхности [2,9,10,13,15]. Выявлено, что до появления концепции об М-клетках в руководствах по гистологии они были известны под названием пещеристых клеток. Однако следует отметить, что цитологическое описание последних не совпадает с имеющейся в литературе характеристикой М-клеток, для которых свойственно наличие в базолатеральных отделах глубоких инвагинаций цитоплазмы, называемых нишами или карманами. Согласно распространенному представлению, в этих цитоплазматических нишах локализируются ассоциированные макрофаги, дендритные клетки и лимфоциты, к которым М-клетки путем трансцитоза доставляют из просвета кишечника различные антигены - макромолекулы и бактерии. Последние, подвергаясь процессингу макрофагами и дендритными клетками, презентуются Т-лимфоцитам, индуцируя тем самым их дифференцировку, что, в свою оче-

редь, сопряжено с активацией В-лимфоцитов. Такова в кратком представлении последовательность событий, которые происходят в фолликул-ассоциированном или купольном эпителии лимфоидных узелков пейеровых бляшек. Согласно данным последних публикаций, в этих процессах оказываются задействованными и другие типы энтероцитов, что в значительной степени усложняет среди них идентификацию М-клеток. Исходя из этого, по мнению некоторых авторов, вопрос о природе и цитологических особенностях этих клеток не может считаться окончательно решенным [1,16,17].

Целью исследования явилось определение специфики микроскопического строения кишечного эпителия, ассоциированного с лимфоидными узелками пейеровых бляшек тонкой кишки белых крыс.

Материал и методы. Исследование проведено на 30 белых крысах-самцах репродуктивного возраста, массой 200,0±20,0 грамм. До этого все животные находились в стандартных условиях экспериментально-биологической клиники (виварий) Украинской медицинской стоматологической академии, согласно правилам содержания экспериментальных животных, установленных Директивой Европейского Парламента и Совета (2010/63/EU), приказом Министерства образования и науки, молодежи и спорта Украины от 01.03.2012 г. №249 «Об утверждении порядка проведения научными учреждениями опытов, экспериментов на животных» и «Общих этических принципов экспериментов на животных», принятых Пятым национальным конгрессом по биоэтике (Киев, 2013) [5,6,12]. Исследование одобрено на заседании Комиссии по биомедицинской этике

при Украинской медицинской стоматологической академии (протокол №155 от 26.04.2017 г.).

После вивисекции, которая осуществлена путем передозировки тиопенталового наркоза (75 мг/кг массы тела животного внутримышечно в верхнюю треть бедра задней лапы [3]), согласно требованиям, предъявляемым к вскрытию брюшной полости, удален весь комплекс желудочно-кишечного тракта и подвергнут фиксации в 10% растворе формалина в течение двух суток. Затем избирательно иссечены короткие отрезки тонкой кишки, в которых находились пейеровы бляшки, выявление которых не представляет труда, так как они отчетливо визуализируются на внешней (внебрыжеечной) поверхности тонкой кишки в виде белесоватых пятен.

Препараты после отмывки от формалина и обезвоживания в спирте возрастающей концентрации заключали в парафиновые блоки и готовили серийные срезы толщиной 4 мкм (Microm HM 325) с дальнейшей окраской их гематоксилином и эозином и по Ван-Гизону, изучение и документирование которых осуществлено посредством светового микроскопа «Konus», оснащенного цифровой микрофотонасадкой Sigeta DCM-900 9.0MP с адаптированной для данных исследований программой Biogex 3 (серийный номер 5604). Морфометрические характеристики тканевых структур соответствующих препаратов получали с помощью системы визуального анализа гистологических препаратов и объект-микрометра Sigeta X 1 мм/100 Div.x0.01мм, масштабная шкала которого составляла 1 мм, а малое деление - 10 мкм и наносилась на соответствующую микрофотографию, полученную при равнозначном увеличении.

Результаты и их обсуждение. Кишечный эпителий, ассоциированный с лимфоидными узелками пейеровых бляшек тонкой кишки, на поперечных гистологических срезах (в поперечном сечении кишечной трубки) визуализируется в довольно разнообразном виде, что, согласно нашим наблюдениям, зависит не только от ракурса сечения, но и от его реактивного состояния. Ниже приводятся иллюстрации выявленных морфологических вариантов: в одних случаях по форме апикальной поверхности лимфоидных узелков он представляется относительно ровным (рис. 1), на других срезах его отличает бугристая форма, состоящая из кластерно расположенных эпителиальных почек (рис. 2).

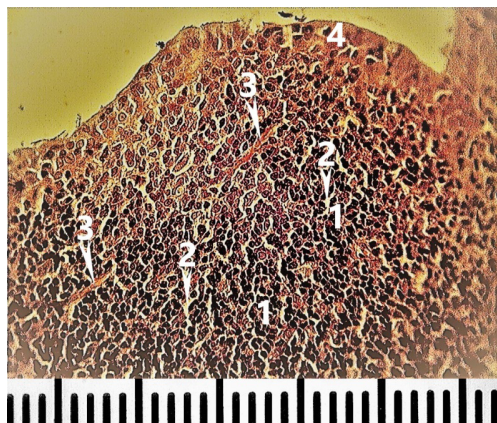


Рис. 1. Апикальный отдел лимфоидного узелка пейеровой бляшки тонкой кишки. Парафиновый срез; окраска по Ван-Гизону; объектив 40. Одно деление масштабной шкалы равно 10 мкм

1 – лимфоидные тяжи, 2 – разделяющие их интерстициальные щели; 3 – соединительнотканые прослойки; 4 – фолликул-ассоциированный эпителий

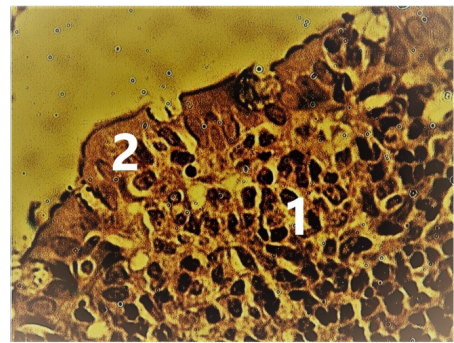


Рис. 2. Апикальные отделы лимфоидных узелков пейеровых бляшек тонкой кишки. Парафиновые срезы; окраска по Ван-Гизону; объектив 100.

1 – лимфоцитарные элементы; 2 – фолликул-ассоциированный эпителий

В процессе изучения серийных срезов удалось обнаружить уникальную форму ассоциации кишечного эпителия с лимфоидной тканью пейеровых бляшек в виде рядного расположения ворсинчатых столбиков, о которых в литературе нет сведений.

В данной структурной форме отчетливо различаются признаки расчленения фолликул-ассоциированного эпителия на дискретные частицы, которые разделены между собой относительно широкими межклеточными щелями (рис. 3). При этом в базальных отделах этих эпителиальных частиц отчетливо выделяются интенсивно базофильные ядра лимфоцитов, которые выстроены в виде коротких лимфоцитарных колонок, теряющихся в извитых тяжах лимфоидной ткани узелка. В полном же охвате данная картина выглядит в виде своеобразного частокола, состоящего из сплоченного ряда колонок, каждая из которых в наглядной форме демонстрирует тесную ассоциацию энтероцитов с лимфоцитарными элементами лимфоидного узелка. Представляется возможность убедиться, что вершину такой колонки занимает один или два энтероцита, ниже от которых столбиками располагаются лимфоцитарные элементы. По мере углубления в толщу лимфоидного узелка, они становятся все более разреженными, теряясь, как было отмечено выше, в извилисто расположенных лимфоидных тяжах. При рассмотрении данных лимфоэпителиальных колонок, толщина которых находится в пределах 10 мкм, обращает внимание, что венчающие их энтероциты отличаются особой формой, заключающейся в наличии на их боковых (апроксимальных) поверхностях углублений, которые сообщаются напрямую с необычно широкими для кишечного эпителия межклеточными щелями, ширина которых иногда достигает 4 мкм (рис. 3). При этом во многих местах видно, что в пограничной зоне лимфоидной ткани эти щели сообщаются с ее интерстициальными прослойками, тогда как со стороны люминальной поверхности они закрыты плотными, замыкающими контактами. Именно эти межклеточные щели придают фолликул-ассоциированному эпителию строго упорядоченную форму в виде лимфоэпителиальных колонок, вершины которых венчаются одним или двумя цилиндрическими энтероцитами. Ввиду наличия у них боковых инвагинаций (карманов) их можно признать М-клетками, однако, согласно существующим представлениям, цитоплазматические карманы М-клеток являются вместилищем для антигенпрезентирующих клеток и лимфоцитов, что совершенно несоизмеримо с шириной наблюдаемых

цитоплазматических инвагинаций. Последнее становится еще более очевидным с учетом того, что поперечный размер указанных энтероцитов, согласно полученным данным, равен всего лишь 10 мкм. Из микрофотографии явствует, что лимфоцитарные элементы находятся в виде колонок под базальными отделами данных энтероцитов (рис. 3).

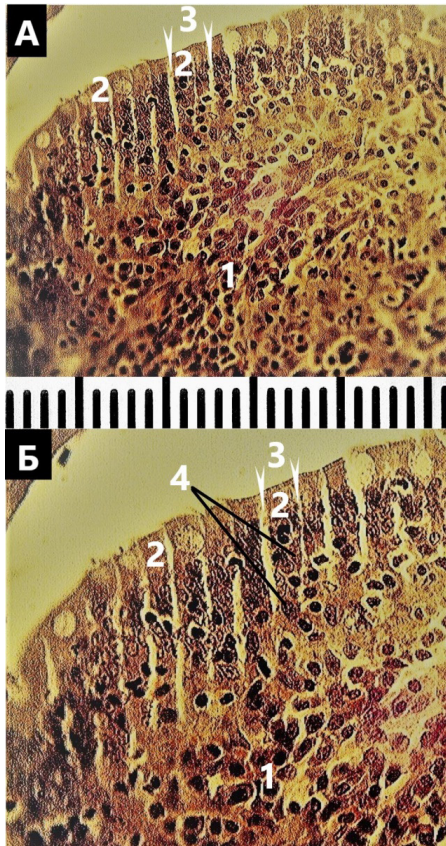


Рис. 3. Апоикальный отдел лимфоидного узелка пейеровой бляшки тонкой кишки. Парафиновый срез; окраска гематоксилином и эозином. А – объектив 40 (одно деление масштабной шкалы – 10 мкм), Б – объектив 100

1 – лимфоидные элементы; 2 – лимфоэпителиальные колонки и 3 – разделяющие их межклеточные щели; 4 – дендритные клетки

Таким образом, последние никаким образом не совпадают с существующими представлениями об М-клетках.

Принимая во внимание, что данная форма организации фолликул-ассоциированного эпителия пейеровых бляшек на гистологических срезах встречается редко, проведено более тщательное, целенаправленное изучение серийных парафиновых срезов, в результате чего обнаружены аналогичные образования, однако в другом, противоположном ракурсе сечения. В данном случае срез выявил их в плоскости, приближенной к люминальной поверхности пейеровой бляшки. На полученной микрофотографии запечатлены пролегающие параллельными рядами клеточные структуры, разделенные светлыми промежутками, в которых в продольном сечении распознаются вышеописанные лимфоэпителиальные колонки (рис. 4).

Следует обратить внимание, что ширина каждого такого клеточного ряда только немногим превышает 10 мкм, а самые широкие щелевые просветы между ними находят-

ся в пределах 4 мкм, что в точности совпадает с их метрической оценкой в колонковой форме. Следовательно, данные лимфоэпителиальные ассоциации не являются в действительности раздельно колонковыми по форме образованиями, а представляют собой параллельно упорядоченные по люминальной поверхности лимфоидного узелка ряды фолликул-ассоциированного эпителия, в связи с чем считаем целесообразным называть их колонковорядными лимфоэпителиальными фракталами (самоподобно повторяющиеся фигуры). По цитологическому составу они представляют собой ряды последовательно соединенных между собой типичных энтероцитов, среди которых, в неравномерном распределении, поодиночке, находятся лимфоцитарные элементы и бокаловидные клетки (рис. 4).

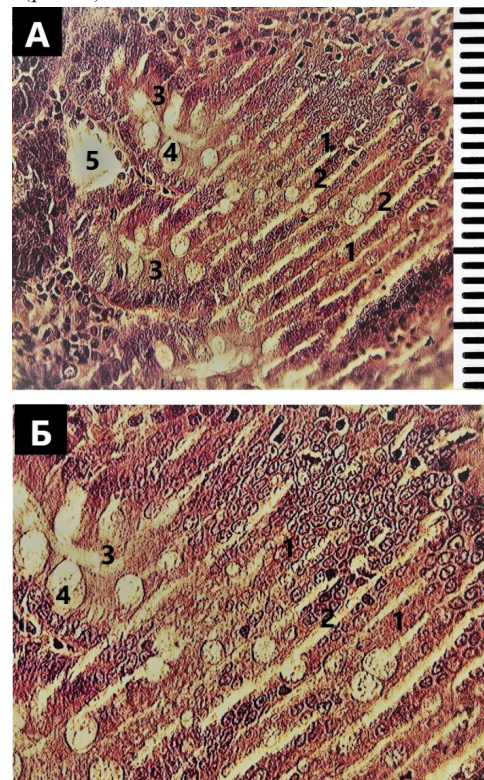


Рис. 4. Тангенциальный (парафиновый) срез лимфоидного узелка пейеровой бляшки тонкой кишки. Окраска гематоксилином и эозином. А – объектив 40 (одно деление масштабной шкалы – 10 мкм), Б – объектив 100

1 – лимфоэпителиальные ряды, 2 – межклеточные щели, разделяющие их; 3 – профильные силуэты внутриузелковых крипт, 4 – их бокаловидные клетки; 5 – поперечный профиль кровеносного сосуда

Следует отметить, что далеко не всегда произвольные гистологические срезы позволяют обнаружить фолликул-ассоциированный эпителий пейеровых бляшек в такой, как представлено выше, строгой конфигурации, что зависит от случайного соотношения между плоскостью среза и конфигурацией тканевых структур в их трехмерном объеме. В подавляющем большинстве случаев при гистологическом изучении лимфоидных узелков пейеровых бляшек их фолликул-ассоциированный эпителий является более привычной по описаниям в литературе формой в виде относительно плоского

или, чаще всего, неровного (почковидного) слоя эпителиальных клеток, примерно так, как это показано в начале описания на рис. 1 и 2. В этом отношении данные исследования полностью совпадают с имеющимися в литературе описаниями, согласно которым фолликул-ассоциированный эпителий пейеровых бляшек рассматривается в качестве монослоя разных по специализации энтероцитов, состоящих преимущественно из абсорбирующих, каемчатых клеток, среди которых кластерно рассредоточены другие их типы и выделяются, прежде всего, бокаловидные клетки, а также эндокриноциты и М-клетки, составляющие 10% от общей популяции [2,8,14].

Проведенные исследования показали, что выявление бокаловидных клеток на гистологических срезах не вызывает никаких затруднений, эндокриноциты идентифицируются с некоторым затруднением, тогда как М-клетки идентифицировать весьма сложно. Их четкому опознанию не помогают даже приводимые многими авторами иллюстрации, на которых чаще всего микрофотографии подменяются интерпретирующими графическими изображениями. В связи с этим самыми надежными методами для идентификации М-клеток являются методы электронной микроскопии [11,16,19].

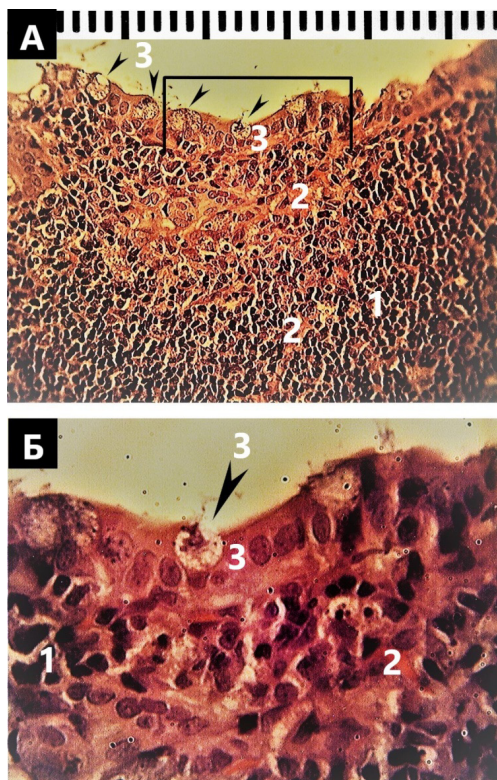


Рис. 5. Апоикальный отдел лимфоидного узелка пейеровой бляшки тонкой кишки. Парафиновый срез; окраска гематоксилином и эозином. А – объектив 40 (одно деление масштабной шкалы – 10 мкм). Прямоугольной скобой указан участок, который представлен на нижней микрофотографии (Б) при большем увеличении (объектив 100)

1 – лимфоцитарные элементы; 2 – соединительнотканые прослойки; 3 – фолликул-ассоциированный эпителий, среди которого находятся бокаловидные клетки с явлениями фагоцитоза (указано стрелками)

Обычные гистологические препараты, на которых в ходе исследования пытались обнаружить цитологические признаки, принадлежащие именно М-клеткам, оказались не состоятельными, в результате того, что многие из этих признаков свойственны и другим типам кишечного эпителия, ассоциированного с лимфоидными узелками. Обращает на себя внимание, что в апоикальных отделах некоторых из бокаловидных клеток имеются явные признаки фагоцитоза какого-то корпускулярного материала (рис. 5). Такое явление довольно распространено и наблюдается в чередующемся порядке по всей апоикальной поверхности лимфоидных узелков. Согласно данным некоторых авторов [7,18,20], фагоцитарными свойствами обладают практически все клетки фолликул-ассоциированного эпителия пейеровых бляшек, что не согласуется с концепцией о М-клетках как об единственных клеточных структурах, осуществляющих посредническую роль между антигенным содержимым кишечника и лимфоидной тканью пейеровых бляшек.

Закключение. Таким образом, результаты проведенного исследования свидетельствуют, что тесное взаимодействие между кишечным эпителием и лимфоидными узелками пейеровых бляшек по структурному воплощению намного многообразнее и сложнее, чем это представлено в литературе. Согласно общепринятой концепции, данное взаимодействие структурно закреплено в тесной связи между непрерывным по протяжению, поляризованным однослойным кишечным эпителием с подлежащими к нему антигенпрезентирующими клетками лимфоидных узелков. При этом в данном фолликул-ассоциированном эпителии среди обычных типов энтероцитов (абсорбирующие, бокаловидные и эндокриноциты) отмечаются особые клетки, наделенные способностью к трансцитозу различных антигенных структур (макромолекулы и микроорганизмы) и передачи их в неизменном виде макрофагам и дендритным клеткам, занимающим место в цитоплазматических карманах этих клеток, известных под названием М-клетки. Следует отметить один существенный изъян в этом представлении, который заключается в том, что трудно себе представить таких размеров цитоплазматические инвагинации (карманы) отдельных энтероцитов, именуемых М-клетками, в которых могли бы помещаться целиком несколько других клеток, осуществляющих процессинг антигенов и презентацию информации о них лимфоцитам, расположенным рядом. Этим замечанием мы не ставим под сомнение данную концепцию об организации фолликул-ассоциированного эпителия и наличия в нем М-клеток. В своих исследованиях стремились наглядно удостовериться в этом. Однако оказалось, что с помощью обычных традиционных гистологических методов, к сожалению, не достигли удовлетворительного результата. Тем не менее, в процессе исследования и досконального знакомства с литературой к решению этой задачи сложился несколько иной подход, который планируется реализовать в дальнейшем.

Наряду с вышепредставленной формой организации фолликул-ассоциированного эпителия пейеровых бляшек, совершенно случайно при изучении серийных парафиновых срезов, нами обнаружена неизвестная по сей день форма ассоциации кишечного эпителия с лимфоидными узелками, которая подробно описана под названием колонковорядных лимфоэпителиальных фракталов. Следует обратить внимание на наличие между ними необычно широких

межклеточных щелей, которые разделяют не только каемчатые энтероциты, но и расположенные под ними столбики лимфоцитарных элементов. Щели явно указывают на прямой парацеллюлярный перенос из кишечного содержимого относительно больших объемов жидкости с растворенными в ней дисперсными веществами. Очевидно, что при ширине в 4 мкм они способны пропускать не только макромолекулы, но и бактерии, на которые в состоянии реагировать соответствующие иммунокомпетентные клетки лимфоцитарных колонок. С этой точки зрения, венчающие их энтероциты не могут претендовать на роль М-клеток, однако в совокупности в составе колонковорядных фракталов они являются фолликул-ассоциированным эпителием.

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SUMMARY

STRUCTURAL FORM OF THE FOLLICLE-ASSOCIATED EPITHELIUM OF PEYER'S PATCHES OF THE ALBINO RATS' SMALL INTESTINE

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The paper was aimed at detailed specification of microscopic structure of the intestinal epithelium, associated with lymphoid nodules of the Peyer's patches of the albino rats' small intestine.

30 mature albino male rats weighted 200,0±20,0 g were involved into the study. Slices of the small intestine with Peyer's patches have been analyzed. Serial paraffin sections have been studied using the "Konus" light microscope. Morphometric characteristics of the tissue structures have been obtained using the Sigeta X 1 mm/100 Div.x0.01mm object-micrometer.

The findings of the study of serial paraffin sections have discovered a hitherto unknown form of association of the intestinal epithelium with lymphoid nodules, which was called column-inline lymphoepithelial fractals. Between them, wide intercellular fissures were found; they separated both the limbic enterocytes, and columns of lymphocytic elements, located beneath them.

Keywords: follicle-associated epithelium, Peyer's patches, lymphoepithelial fractals.

РЕЗЮМЕ

СТРУКТУРНЫЕ ФОРМЫ ФОЛЛИКУЛ-АССОЦИИРОВАННОГО ЭПИТЕЛИЯ ПЕЙЕРОВЫХ БЛЯШЕК ТОНКОЙ КИШКИ БЕЛЫХ КРЫС

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Целью исследования явилось определение специфики микроскопического строения кишечного эпителия, ассоциированного с лимфоидными узелками пейеровых бляшек тонкой кишки белых крыс.

Исследование проведено на 30 белых крысах-самцах репродуктивного возраста, массой 200,0±20,0 грамм. Материалом для изучения служили участки тонкой кишки с пейеровыми бляшками. Под световым микроскопом «Konus» изучены серийные парафиновые срезы. Морфометрические характеристики тканевых структур получали с помощью объект-микрометра Sigeta X 1 мм/100 Div.х0.01 мм.

При изучении серийных парафиновых срезов обнаружена неизвестная по сей день форма ассоциации кишечного эпителия с лимфоидными узелками, которая названа колонковорядными лимфоэпителиальными фракталами. Между ними обнаружены широкие межклеточные щели, которые разделяют не только каемчатые энтероциты, но и расположенные под ними столбики лимфоцитарных элементов.

რეზიუმე

თეთრი ვირთაგვების წვრილი ნაწლავის პეიერის ფოლაქების ფოლიკულ-ასოცირებული ეპითელიუმის სტრუქტურული ფორმები

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უკრაინის სამედიცინო სტომატოლოგიური აკადემია, ¹ადამიანის ანატომიის კათედრა; ²კლინიკური ანატომიისა და ოპერაციული ქირურგიის კათედრა, პოლტავა, უკრაინა

კვლევის მიზანს წარმოადგენდა თეთრი ვირთაგვების წვრილი ნაწლავის პეიერის ფოლაქების ფოლიკულ-ასოცირებული ეპითელიუმის სტრუქტურული ფორმების სპეციფიკის განსაზღვრა.

კვლევა ჩატარდა რეპროდუქციული ასაკის 30 თეთრ მამრ ვირთაგვებზე წონით 200.0±20.0 გრ. კვლევის მასალას წარმოადგენდა წვრილი ნაწლავის ნაწილები პეიერის ფოლაქებით. პარაფინის სერიული ანათლები შესწავლილია «Konus»-ის სხივური მიკროსკოპის მეშვეობით. ქსოვილის სტრუქტურების მორფომეტრიული მახასიათებლები მიღებულია Sigeta X1მმ/100 Div. x0.01 მმ ობიექტ-მიკრომეტრის გამოყენებით. პარაფინის სერიული ანათლების შესწავლის შედეგად გამოვლინდა ნაწლავის ეპითელიუმის ლიმფოიდურ კვანძებთან ასოცირებული, დღემდე უცნობი, ფორმა, რომელიც წოდებულია სვეტურ-რიგის ლიმფოციტული ფრაქტალებად. მათ შორის აღმოჩენილია ფართო უჯრედშორისი ნაპრალი, რომელიც ყოფს არა მხოლოდ შემოსაზღვრულ ენტეროციტებს, არამედ მათ ქვეშ მდებარე ლიმფოციტურ ელემენტების სვეტებსაც.

DISTRIBUTION OF INTRAEPITHELIAL LYMPHOCYTES AND MACROPHAGES IN CERVICAL MICROENVIRONMENT DURING THE PROGRESSION OF CERVICAL INTRAEPITHELIAL NEOPLASIA

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Cervical cancer represents second most common cancer after breast cancer in Georgian women [1]. Cervical cancer develops from pre-cursor lesions collectively named as cervical intraepithelial neoplasia (CIN). There are three different grades of CIN, including CIN1, CIN2 and CIN3 [2]. Histologically CIN1 is characterised with maturation of upper two thirds of epithelium, slight and variable cellular and nuclear atypia and sometimes with viral cytopathic effect (koilocytosis). Mitotic figures might be present in lower third of the epithelium. However, pathological mitoses are not detected. In case of CIN2, maturation is detected in upper half of the epithelium. Nuclear atypia is present in both halves of the epithelium and usually mitotic figures are present in lower two thirds of epithelium. Pathological mitoses might be present. In CIN3 epithelial maturation may not be detected. Nuclear atypia is present in whole thickness of epithelium,

mitotic figures are numerous and detected through the epithelium. Pathological mitoses are common [2]. The major risk factor of the development of cervical intraepithelial neoplasia and cervical carcinoma is human papilloma virus (HPV). There are more than 100 HPV subtypes, from which only part of them, so called high-risk HPV types can cause the cancer. These high-risk HPV types, including HPV 16 and 18 are characterised with the expression of two types of early genes, named E6 and E7, which play the major role in cervical carcinogenesis.

Developing tumors, including CIN and cervical cancer are surrounded by tumor microenvironment (TME), represented by tumor stroma, tumor vasculature, tumor infiltrating lymphocytes, macrophages and other tumor associated cells. TME is a unique milieu, which is developed together with the progression of tumor, as a result of the interaction with host organism. TME

is formed by tumor itself. Immune cells in TME include the components of both innate and adaptive immunity, such as macrophages, dendritic cells, T cells, or so called tumor infiltrating lymphocytes and natural killer (NK) cells [3]. It has been shown that HPV virus regulates various aspects of innate and adaptive immune responses in precancerous and cancerous cervical lesions. Macrophages and T lymphocytes play the major role against the infection with high risk HPV types [4]. Recent study from Chen et al., showed that CD68+ macrophages are associated with the high risk HPV infection. In addition the number of CD68+ macrophages in increased along with the progression of CIN to invasive cancer [5]. CD103 marker is widely expressed in intraepithelial T lymphocytes and dendritic cells. It has been shown that CD103 is expressed in CD8+ cytotoxic T cells from the peripheral blood of cervical cancer patients [6]. However, the role of CD103 expression in pre-cancerous and cancerous cervical lesions itself is not yet studied. The aim of our study was to investigate the distribution of CD68+ macrophages and CD103 intraepithelial lymphocytes during the progression of CIN and cervical carcinoma *in situ*.

Material and methods. *Tissue samples.* Archival formalin-fixed and paraffin-embedded (FFPE) tissue samples, diagnosed as CIN or *in situ* CA, between 2015-2018 years, were obtained from the department of pathological anatomy, N. Kipshidze central university clinic, Tbilisi, Georgia. Study cohort included 20 cases with normal cervical tissue, 31 cases of CIN1, 24 cases of CINII, 26 cases of CINIII and 42 cases of *in situ* carcinoma (CA), and 35 cases of invasive cervical carcinoma (CA), altogether 178 cases. Specimens of lesions were obtained from cervical biopsies, cone biopsies, loop electrosurgical excision procedure and radical hysterectomy. Normal cervical samples were obtained from hysterectomy, due to benign conditions, without a history of CIN or abnormal Pap smears. Standard Haematoxylin and Eosin (H&E) stained sections were revised by two independent pathologists (T.M. and G.B.). From 31 CIN1 cases, 8 cases were further progressed in CIN2 and from original 24 CIN2 cases, 12 cases were progressed into CIN3 or *in situ* CA. The age of patients varied from 30 to 50 years.

Tumor infiltrating lymphocytes (TILs) were analysed on standard H&E stained sections, in three areas of the tumor, including tumor bead, invasive tumor margin and tumor associated stroma by semi-quantitative approach. TILs were classified as following, no infiltration (0), low infiltration (1), moderate infiltration (2) and high infiltration (3).

4 μ FFPE tissue sections were deparaffinized in xylene and rehydrated by using serial dilutions of ethanol (96%, 80%, 70%) and heat mediated antigen retrieval has been performed. Antibodies against the following antigens were used: Ki67, CD103, CD68. Lymphocyte and macrophage counts were analysed in 20 high power fields (HPF) per case. In addition, for CD103 positive intraepithelial lymphocytes the lympho-epithelial index was calculated, meaning the ratio between per 100 epithelial cells and infiltrating CD103 positive T cells. Lymphocyte proliferation index was calculated as low, meaning ≤ 10 proliferating lymphocytes and high, meaning >10 proliferating lymphocytes labelled by Ki67.

A comparison between different groups has been performed by the use of Kruskal-Wallis test and non-parametric correlations have been estimated by Spearman's rank test. In all tests, p values < 0.05 considered as significant. Statistical analysis of data has been performed using SPSS 19 statistical program.

Results and their discussion. From 20 cases of normal cervical epithelium, no infiltrate was present in 10/20 (50%) cases,

low infiltrate was present in 6/20 (30%) cases, moderate infiltrate was present 4/20 (20%) cases. In CIN1, no infiltrate was present in 8/31 (25.8%) cases, low infiltrate was present in 13/31 (41.9%) cases, moderate infiltrate was present 6/31 (19.35%) cases and high infiltration was present in 4/31 (12.9%) cases. In CIN2, no infiltrate was present in 2/24 (8.3%) cases, low infiltrate was present in 9/24 (37.5%) cases, moderate infiltrate was present 7/24 (29.1%) cases and high infiltration was present in 6/24 (25%) cases. In CIN3, no infiltrate was present in 1/26 (3.8%) cases, low infiltrate was present in 7/26 (26.9%) cases, moderate infiltrate was present 10/26 (38.5%) cases and high infiltration was present in 9/26 (34.6%) cases. In *in situ* CA, no infiltrate was present in 0/42 (0%) cases, low infiltrate was present in 8/42 (19%) cases, moderate infiltrate was present 15/42 (35.7%) cases and high infiltration was present in 19/42 (45.2%) cases. In invasive CA, no infiltrate was present in 0/35 (0%) cases, low infiltrate was present in 9/35 (27.7%) cases, moderate infiltrate was present 12/35 (34.2%) cases and high infiltration was present in 14/35 (40%) cases. From eight CIN1 patients which progressed into CIN2 lesion 5/8 (62.5%) was characterised with the absence of immune infiltrate, 2/8 (25%) cases were characterised with the presence of low infiltrate and 1/8 (12.5%) case was characterised with moderate infiltrate. From 12 CIN2 cases, which were progressed into CIN3 or *in situ* CA, 2/12 (16.6%) cases were characterised with the absence of the immune infiltrate, 7/12 (58.3%) cases were characterised with the low immune infiltration and 3/12 (25%) cases were characterised with moderate immune infiltration.

From 20 cases of normal cervical epithelium, LEi < 0.1 was present in 17/20 (85%) cases and LEi 0.1-0.5 was present in 3/20 (15%) of cases. In CIN1 LEi < 0.1 was present in 25/31 (80.6%) cases, LEi 0.1-0.5 was present in 4/31 (12.9%) cases and LEi > 0.5 was present in 2/31 (6.5%) cases. In CIN2 LEi < 0.1 was present in 16/24 (66.7%) cases, LEi 0.1-0.5 was present in 5/24 (20.8%) cases and LEi > 0.5 was present in 3/24 (12.5%) cases. In CIN3 LEi < 0.1 was present in 9/26 (34.6%) cases, LEi 0.1-0.5 was present in 10/26 (38.5%) cases and LEi > 0.5 was present in 7/26 (26.9%) cases. In *in situ* CA LEi < 0.1 was present in 13/42 (30.9%) cases, LEi 0.1-0.5 was present in 15/42 (35.7%) cases and LEi > 0.5 was present in 12/42 (28.5%) cases. In invasive CA LEi < 0.1 was present in 9/35 (25.7%) cases, LEi 0.1-0.5 was present in 14/35 (40%) cases and LEi > 0.5 was present in 12/35 (34.2%) cases. From 8 CIN1 patients which progressed into CIN2 lesion all 8 cases were characterised with the LEi of < 0.1 . From 12 CIN2 cases, which were progressed into CIN3 or *in situ* CA, 6/12 (50%) cases were characterised with the LEi of < 0.1 , 4/12 (33.3%) cases were characterised with LEi of 0.1-0.5 and 2/12 (16.6%) cases were characterised with the LEi of > 0.5 .

In all cases of normal cervix with lymphocyte infiltration (n=10), the lymphocyte proliferation index was low, meaning ≤ 10 Ki67 positive lymphocytes. In CIN1, from all cases with lymphocyte infiltration (n=23), eight (34.7%) cases were with low lymphocyte proliferation index and 15 (65.2%) cases were with high lymphocyte proliferation index, meaning Ki67 positive lymphocytes > 10 . In CINII from all cases with lymphocyte infiltration (n=22), low lymphocyte proliferation index was detected in 6 (27.2%) cases, whilst 16 (72.7%) cases were characterised with high lymphocyte proliferation index. In CINIII from all cases with lymphocyte proliferation (n=26), low lymphocyte proliferation index was detected in 16 (61.5%) cases, whilst high lymphocyte proliferation index was detected in 10 (38.5%) cases. In *in situ* CA, from all cases with lymphocyte infiltration (n=42), 25 (59.5%) cases were characterised with low lymphocyte proliferation index and 17 (40.5%) cases

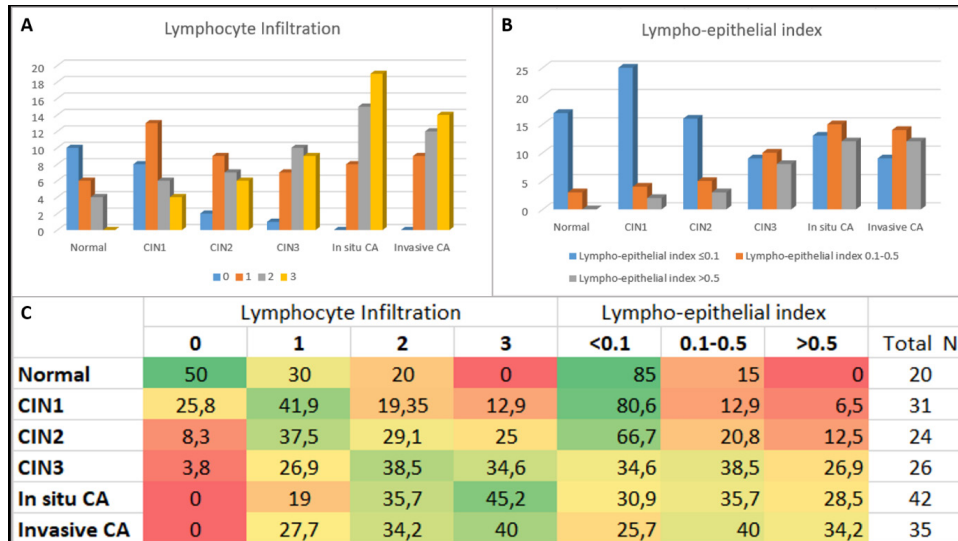


Fig. 1. A. Distribution of immune infiltrate in cervical intraepithelial neoplasia and in carcinoma, B. Distribution of lympho-epithelial index in cervical intraepithelial neoplasia and in carcinoma (C) heat map of percentages, showing the distribution of lymphocyte infiltration and lympho-epithelial index in cervical intraepithelial neoplasia and in carcinoma, red squares represent lowest percentages and green represent highest percentages, whilst yellow colours represent moderate percentages.
0, no infiltration, 1, low infiltration, 2, moderate infiltration, 3, high infiltration

	Lymphocyte Count				Lympho-epithelial index			Lymphocyte proliferation Index		Total N
	0	1	2	3	<0,1	0.1-0.5	>0.5	≤10	>10	
CINI to CINII	6	2	1	0	8	0	0	3	0	8
CINII to CINIII or CA	2	7	3	0	6	4	2	10	0	12

Fig. 2. The distribution of lymphocyte count, lympho-epithelial index and lymphocyte proliferation index in patients with progressive disease. CA, carcinoma

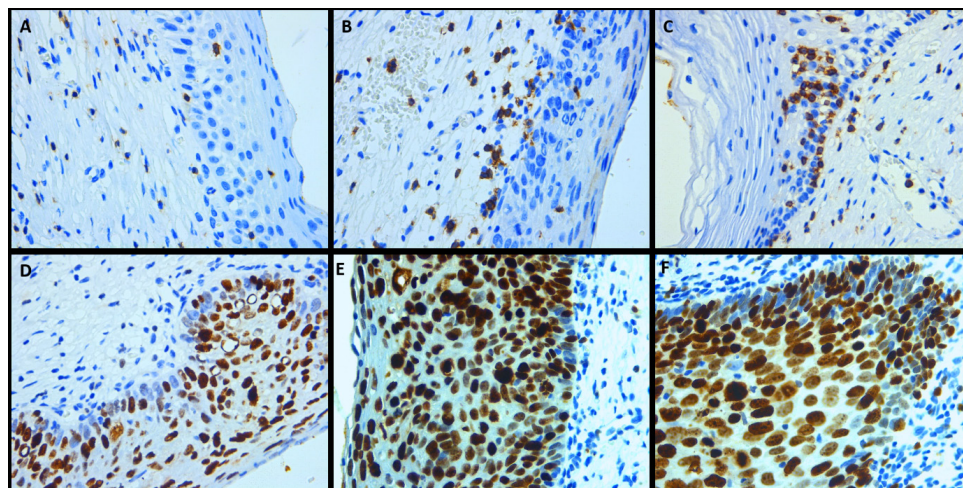


Fig. 3. Representative examples of A. lympho-epithelial index <0.1, B. lympho-epithelial index 0.1-0.5, C. lympho-epithelial index >0.5, D.E. lymphocyte proliferation index low and F. lymphocyte proliferation index high

were characterised with high lymphocyte proliferation index. In invasive CA, from all cases with lymphocyte infiltration (n=35), 22 (62.9%) cases were characterised with low lymphocyte infiltration index and 13 (37.1%) cases were characterised with high lymphocyte proliferation index. From 8 cases of CIN1, which were progressed to CINII, only 3 cases were characterised with the presence of immune infiltrate and all of them were characterised with the absence of Ki67 in lymphocytes. In 12 cases of CINII which was

progressed to CINIII or in carcinoma, 10 cases were characterised with immune infiltrate, from which in all cases lymphocyte proliferation index was low.

In normal epithelia mean macrophage count was 16.50±5.2, in CIN1 mean macrophage count was 39.70±5.6, in CINII mean macrophage count was 51.89±5.9, in CINIII mean macrophage count was 69.45±4.5 in situ CA mean macrophage count was 89.78±6.04 and in invasive CA mean macrophage count was 121.07±8.4.

We have analysed the microenvironment alterations in different degrees of cervical intraepithelial neoplasia, including 8 CIN1 cases, which progressed into CINII, and 12 CINII cases, which were progressed into CINIII or carcinoma. Microenvironment alterations in CIN were compared to normal cervical epithelia and carcinoma in situ and invasive carcinoma.

First, we have analysed the distribution of lymphocytes in haematoxylin and eosin stained sections from different grades of CIN, normal cervix, in situ CA and invasive CA. We have collectively evaluated immune infiltrates in three areas of the tumor, including tumor bead, invasive tumor margin and tumor associated stroma by semi quantitative approach. We employed 4 tiered grading system, particularly; immune infiltrates were classified as following, no infiltration (0), low infiltration (1), moderate infiltration (2) and high infiltration (3). We have found that 50% of normal cervical epithelia is characterised with various degrees of immune infiltrate, whilst collectively 74.2% cases of CIN1 is characterised with various degrees of immune infiltrate, from which high infiltration is present in 12.9% of cases. The occurrence of high infiltration is increased in CINII and CINIII representing 25% and 34.6% respectively and highest infiltration has been seen in patients with in situ CA (45.2%). Bedoya et al., also found that the density of CD8+ T lymphocytes is higher in carcinoma, compared to CINIII lesions [7]. These data collectively suggest that immune infiltrates are significantly increased in higher grades of CIN lesions and in situ and invasive CA, which might be due to the activation of innate and adaptive immune system. However, not in all cases immune infiltrate is able to stop the progression of the disease, which might be due to some innate and adaptive immune defects. In line with this hypothesis, we have found that from 8 CIN1 patients which progressed into CIN2 lesion 62.5% was characterised with the absence of immune infiltrate, 25% cases were characterised with the presence of low infiltrate and 12.5% cases were characterised with moderate infiltrate. From 12 CIN2 cases, which were progressed into CIN3 or in situ CA, 16.6% cases were characterised with the absence of the immune infiltrate, 58.3% cases were characterised with the low immune infiltration and 25% cases were characterised with moderate immune infiltration.

For more detailed characterisation of immune infiltrates, we have analysed the expression of CD103 T lymphocyte marker. CD103 has been recently found to be specifically expressed in intraepithelial T lymphocytes and CD103 positive intraepithelial T lymphocytes has been found to be increased in cervical lesions. They are also associated with prognostic impact in cervical cancer, according to Komdeur and colleagues [8] not all tumor-infiltrating T cells confer equal benefit to patients, with epithelial T cells being superior to stromal T cells. To assess whether the epithelial T cell biomarker CD103 could specifically discriminate the beneficial antitumor T cells, association of CD103 with clinicopathological variables and outcome was analyzed in the TCGA cervical cancer data set (n = 304). To the best of our knowledge, we are first who implemented the lympho-epithelial index based on the expression of CD103 in cervical epithelia in different types of cervical precancerous and cancerous lesions. We calculated lympho-epithelial index as the ratio between per 100 epithelial cells and infiltrating CD103 positive T cells. We divided LEi into three grades, such as LEi <0.1, LEi = 0.1-0.5 and LEi >0.5. LEi <0.1 was present in 85% cases of normal cervical epithelium and LEi 0.1-0.5 was present in 15% of cases of normal cervical epithelium. In CIN1 LEi <0.1 was present in 80.6% cases, LEi 0.1-0.5 was present in 12.9% cases and LEi >0.5 was present in 6.5% cases. In CIN2 LEi <0.1 was

present in 66.7% cases, LEi 0.1-0.5 was present in 20.8% cases and LEi >0.5 was present in 12.5% cases. In CIN3 LEi <0.1 was present in 34.6% cases, LEi 0.1-0.5 was present in 38.5% cases and LEi >0.5 was present in 26.9% cases. In in situ CA LEi <0.1 was present in 30.9% cases, LEi 0.1-0.5 was present in 35.7% cases and LEi >0.5 was present in 28.5% cases. In invasive CA LEi <0.1 was present in 25.7% cases, LEi 0.1-0.5 was present in 40% cases and LEi >0.5 was present in 34.2% cases. Collectively our data shows that, similarly to immune infiltrates, higher LEi has been seen in higher grades of CIN and in situ and invasive CA. Interestingly from 8 CIN1 patients which progressed into CIN2 lesion all 8 cases were characterised with the LEi of <0.1. However, from 12 CIN2 cases, which were progressed into CIN3 or in situ CA, only 50% cases were characterised with the LEi of <0.1, whilst 33.3% cases were characterised with LEi of 0.1-0.5 and 16.6% cases were characterised with the LEi of >0.5. This again indicates the functional deficiency in lymphocytes and inability to perform their cytotoxic action.

To gain more insight in the functionality of immune infiltrates, we have analysed the lymphocyte proliferation index using Ki67 labelling. In all cases of normal cervix with lymphocyte infiltration the lymphocyte proliferation index was low, meaning ≤ 10 Ki67 positive lymphocytes. In CIN1, from all cases with lymphocyte infiltration 34.7% cases were with low lymphocyte proliferation index and 65.2% cases were with high lymphocyte proliferation index, meaning Ki67 positive lymphocytes >10. In CINII from all cases with lymphocyte infiltration, low lymphocyte proliferation index was detected in 27.2% cases, whilst 72.7% cases were characterised with high lymphocyte proliferation index. In CINIII from all cases with lymphocyte proliferation, low lymphocyte proliferation index was detected in 61.5% cases, whilst high lymphocyte proliferation index was detected in 38.5% cases. In situ CA, from all cases with lymphocyte infiltration 59.5% cases were characterised with low lymphocyte proliferation index and 40.5% cases were characterised with high lymphocyte proliferation index. In invasive CA, from all cases with lymphocyte, infiltration 62.9% cases were characterised with low lymphocyte infiltration index and 37.1% cases were characterised with high lymphocyte proliferation index. From eight cases of CIN1, which were progressed to CINII, only three cases were characterised with the presence of immune infiltrate and all of them were characterised with the absence of Ki67 in lymphocytes. In 12 cases of CINII, which was progressed to CINIII, or in carcinoma, 10 cases were characterised with immune infiltrate, from which in all cases lymphocyte proliferation index was low. Our data suggest that lymphocyte proliferation index is the important indicator of the functionality of lymphocytes and might be further used in evaluation of the functionality of lymphocytes and the assessment of the risk of the progression of CIN lesions. To the best of our knowledge, we are also first who implemented the evaluation lymphocyte proliferation index in cervical precancerous and cancerous lesions.

In addition, we have also analysed the infiltration with CD68 positive macrophages. CD68 positive macrophages represent the first arm of the innate immune system [9]. However, CD68 labelling defines general monocyte-macrophage lineage and does not differentiate between different types of macrophages, such as anti-tumorigenic M1 and pro-tumorigenic M2 macrophages [10]. Nevertheless, previous study from Chen et al., showed that CD68 macrophages were positively associated with the infection of high risk HPV and also they were increased in the progression of cervical disease [5]. In line with the findings with Chen et al., we have found that the number of CD68 positive macrophages is significantly increased in higher grades of CIN and cervical carcinoma, however, we did not find any

relationship with the direct progression of CIN. Which might be explained by the fact that, CD68 does not differentiate protumorigenic and anti-tumorigenic macrophages.

Conclusions. Immune infiltrates as well as lympho-epithelial index is significantly increased in higher grades of CIN and in situ and invasive CA. Evaluation of lympho-epithelial index is superior compared to the evaluation of the raw immune infiltrates in the assessment of the risk of the CIN progression, and it might be used for the assessment of the risk of CIN progression. In addition, the complex analysis together with lymphocyte proliferation index might represent more functional analytical approach to evaluate the efficiency of anti-tumor immune response and therefore to estimate the risk of progression of CIN lesions.

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SUMMARY

DISTRIBUTION OF INTRAEPITHELIAL LYMPHOCYTES AND MACROPHAGES IN CERVICAL MICROENVIRONMENT DURING THE PROGRESSION OF CERVICAL INTRAEPITHELIAL NEOPLASIA

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Microenvironment plays central role in the development of cervical precancerous and cancerous lesions. Cervical intraepi-

thelial neoplasia (CIN) represents a group of precancerous lesions, divided into three degrees.

We investigated the distribution of intraepithelial lymphocytes and macrophages in different grades of CIN. We analysed lymphocyte marker CD103, macrophage marker CD68 and proliferation marker Ki67 using standard immunohistochemistry. In addition, we investigated the distribution of lymphocytes using standard haematoxylin and eosin method. The results of our study indicated that grade I CIN which subsequently progressed into grade II CIN was characterised with low lymphocytic infiltration, low lympho-epithelial index and low lymphocyte proliferation index. Similar results were seen in cases of CINII which were later progressed into CINIII or in carcinoma.

Therefore, we would like to recommend the analysis of microenvironment alterations in CIN lesions, in order to assess their progression potential.

Keywords: cervical intraepithelial neoplasia, intraepithelial lymphocytes, macrophages.

РЕЗЮМЕ

ОСОБЕННОСТИ РАСПРЕДЕЛЕНИЯ ИНТРАЭПИТЕЛИАЛЬНЫХ ЛИМФОЦИТОВ И МАКРОФАГОВ В МИКРОСРЕДЕ ШЕЙКИ МАТКИ В ПРОЦЕССЕ ПРОГРЕССИИ ЕЕ ИНТРАЭПИТЕЛИАЛЬНОЙ НЕОПЛАЗИИ

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Микросреда играет значимую роль при предопухолевых поражениях и в процессе образования опухолевых заболеваний. Интраэпителиальная неоплазия шейки матки является предопухолевым процессом, который в зависимости от сложности делится на 3 степени.

Целью исследования явилось изучение особенностей распределения интраэпителиальных лимфоцитов и макрофагов при интраэпителиальных неоплазиях шейки матки различной степени.

Стандартным иммуногистохимическим методом изучены молекулярные маркеры: лимфоцитарный маркер CD103, макрофагальный маркер CD68 и пролиферативный маркер Ki67. Кроме того изучено распределение лимфоцитов в стандартных препаратах, окрашенных гематоксилином и эозином.

Результаты исследования выявили, что интраэпителиальная неоплазия I степени, которая прогрессировала в интраэпителиальную неоплазию II степени характеризовалась низкими показателями лимфоцитарной инфильтрации, лимфо-эпителиального индекса и пролиферативного индекса лимфоцитов. Аналогичными оказались результаты и при интраэпителиальных неоплазиях II степени, которые прогрессировали в интраэпителиальную неоплазию III степени или карциному. В результате проведенного исследования авторы рекомендуют оценивать изменения микросреды при предопухолевых поражениях шейки матки с целью определения риска их прогрессии.

რეზიუმე

ინტრაეპითელური ლიმფოციტების და მაკროფაგების განაწილების თავისებურებები საშილოსნოს ყელის მიკროგარემოში მისი ინტრაეპითელური ნეოპლაზიის პროგრესის პროცესში

ქ.მანჯგალაძე, გ.თევდორაშვილი, თ.ალიბეგაშვილი
მ.გაჩეჩილაძე, გ.ბურკაძე

თბილისის სახელმწიფო სამედიცინო უნივერსიტეტი,
საქართველო

მიკროგარემო მნიშვნელოვან როლს თამაშობს სიმსივნისწინარე დაზიანებებისა და სიმსივნური პროცესების ჩამოყალიბების დროს. საშილოსნოს ყელის ინტრაეპითელური ნეოპლაზია წარმოადგენს სიმსივნისწინარე პროცესს, რომელიც განვითარების სიმპომის გათვალისწინებით იყოფა სამ ხარისხად.

კვლევის მიზანს წარმოადგენდა ინტრაეპითელური ლიმფოციტებისა და მაკროფაგების განაწილების თავისებურებების შესწავლა საშილოსნოს ყელის სხვა-

დასხვა ხარისხის ინტრაეპითელურ ნეოპლაზიებში. სტანდარტული იმუნოჰისტოქიმიური მეთოდით გამოკვლეულია CD103 ლიმფოციტების, CD68 მაკროფაგების და Ki67 პროლიფერაციის მარკერები. შესწავლილია ლიმფოციტების განაწილება სტანდარტულ ჰემატოქსილინით და ეოზინით შეღებილ ანათლებში.

კვლევის შედეგებმა გამოავლინა, რომ საშილოსნოს ყელის I ხარისხის ინტრაეპითელური ნეოპლაზია, რომელმაც შემდგომში პროგრესია განიცადა II ხარისხის ინტრაეპითელურ ნეოპლაზიად ხასიათდება მწირი ლიმფოციტური ინფილტრატით, დაბალი ლიმფოციტური ინდექსით და ლიმფოციტების დაბალი პროლიფერაციული ინდექსით. ანალოგიური შედეგები გამოვლინდა საშილოსნოს ყელის II ხარისხის ინტრაეპითელური ნეოპლაზიის იმ შემთხვევებშიც, რომელთაც განიცადეს პროგრესია III ხარისხის ინტრაეპითელურ ნეოპლაზიად ან კარცინომად.

ავტორების მიერ რეკომენდებულია საშილოსნოს ყელის სიმსივნისწინარე დაზიანებებში მიკროგარემოს ცვლილებების შეფასება მათი პროგრესიის რისკის განსაზღვრის მიზნით.

EPIGENETIC CHANGES – HISTONE 3 PHOSPHORYLATION – EPITHELIAL OVARIAN TUMORS

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Epigenetic modifications in normal and cancer cells represent the phenotypic alterations in gene expression, without the change in DNA sequence. Epigenetics plays an important role in oncological diseases [1]. However, its role in the improvement of the ovarian cancer management is not yet clear. DNA methylation represents the most well studied mechanism of epigenetic modification. Hypermethylation, results in the repression of gene expression whilst hypomethylation results in gene expression. Another important mechanism of epigenetic regulation is histone modifications [1]. Histone octamer, composed of four major histones, H2A, H2B, H3 and H4 create nucleosomes, which represent the major building block of chromatin. Histone tails represent the sites of reversible modifications, which alter chromatin structure and therefore gene expression. Most common histone modifications are acetylation, methylation, ubiquitination and phosphorylation [2]. All four nucleosomal histone tails can be phosphorylated by different protein kinases and dephosphorylated by phosphatases [2]. Histone phosphorylation occurs in serine, threonine and tyrosine residues [2]. The most well-known histone phosphorylation event is the phosphorylated histone H2A(X) which occurs during DNA damage. This modification marks DNA double strand breaks and makes DNA accessible to different DNA repair factors [3]. Another, important phosphorylation event is the phosphorylation of histone-H3 in serine 10th and 28th residues. Phosphorylation of histone H3 attracted an interest since it was demonstrated to closely associ-

ate with condensation of mitotic chromosomes in mammalian cells. Although this correlation has been observed during mitosis and meiosis in a wide range of eukaryotes, the functional significance of this modification at individual sites of histone H3 differs among species. Mitotic histone H3 phosphorylation in mammalian cells occurs massively at several residues, including serines 10 and 28 [4]. Phosphorylation of Ser10 in histone H3 has been explicitly studied in many organisms. This modification is involved in both transcription and cell division, which are two opposite events. In interphase phosphorylation of H3 correlates with chromatin relaxation and gene expression, whilst in mitosis it correlates with chromatin condensation [5].

pHH3 antibodies specifically detect the core protein histone H3 only when phosphorylated at serine 10 (Ser10) or serine 28 (Ser28). Immunohistochemistry (IHC) for pHH3 has been used for mitotic cell counting in different types of tumours as marker of cells in late G2 and M phases. Several recent studies also showed its prognostic value in various cancer types. Elevated expression of pHH3 has been detected in glioma tissues, in prostate carcinoma, in breast cancer and in gastrointestinal stromal tumors [6]. The aim of our study was to investigate the distribution of pHH3 in different epithelial tumors of the ovary, and to analyse its potential relationship with ER, PR, Ki67, p53 and BCL2.

Material and methods. Study included altogether 160 patients, diagnosed between the years of 2015 – 2018, from which

20 cases were serous cystadenomas (group I), 20 cases were mucinous cystadenomas (group II), 20 cases - serous borderline tumors (BOTs) (group III), 20 cases – mucinous borderline tumors (BOTs) (group IV), 20 cases – low grade serous adenocarcinomas (group V), 20 cases – low grade mucinous adenocarcinomas (group VI), 20 cases – high grade serous adenocarcinomas (group VII) and 20 cases – high grade mucinous adenocarcinomas (group VIII). The age of patients was ranged from 10 to 80, with the median age of 56.

Formalin fixed and paraffin embedded surgical tissue material were retrieved from the archives of the department of pathology, N. Kipshidze central university clinic, Tbilisi, Georgia. Routine haematoxylin and eosin stained slides were revised and classified by two independent pathologists (V.M., T.M.). Alcian-blue staining was used to distinguish serous BOTs from mucinous BOTs (the latter is positive for Alcian-blue staining). In addition, the number of mitotic figures were evaluated in 20 high power fields (x200).

4µ FFPE tissue sections were deparaffinized in xylene and rehydrated by using serial dilutions of ethanol (96%, 80%, 70%) and heat mediated antigen retrieval has been performed. Antibodies against the following antigens were used: WT1 (WT49); ER (6F11); PR (NCL-L-PGR-312, clone 16), BCL2 Ki67(MM1); P53 (DO7) and phosphohiston-H3 (PHH3) Immunohistochemical staining was evaluated in 20 high power fields (x200) for each marker in each case and the average number of positive cells were recorded.

Comparisons between different groups has been performed by the use of Kruskal-Wallis test and non-parametric correlations have been estimated by Spearman's rank test. In all tests, p values ≤ 0.05 considered as significant. Statistical analysis of data has been performed using SPSS 19 statistical program.

Results and their discussion. Mitotic counts. Mitotic figures were not detected in first two groups of patients, including serous cystadenomas (group I) and mucinous cystadenomas (group II). In groups III and IV serous BOTs and mucinous BOTs, the mitotic figures were detected in 4/20 (20%) and 6/20 (30%) cases respectively. In groups V and VI, low grade serous carcinoma and low grade mucinous carcinoma mitotic figures were detected in 9/20 (45%) and 11/20 (55%) cases respectively and in groups VII and VIII, high grade serous carcinoma and high grade mucinous carcinoma mitotic figures were detected in 14/20 (75%) and 16/20 (85%) cases respectively.

Expression of phosphohiston-H3 in groups. PHH3 was not detected in first two groups of patients, including serous cystadenomas (group I) and mucinous cystadenomas (group II). In groups III and IV serous BOTs and mucinous BOTs, the mitotic figures were detected in 6/20 (30%) and 8/20 (40%) cases respectively. In groups V and VI, low grade serous carcinoma and low grade mucinous carcinoma mitotic figures were detected in 15/20 (75%) and 13/20 (65%) cases respectively and in groups VII and VIII, high grade serous carcinoma and high grade mucinous carcinoma mitotic figures were detected in 17/20 (85%) and 19/20 (90%) cases respectively.

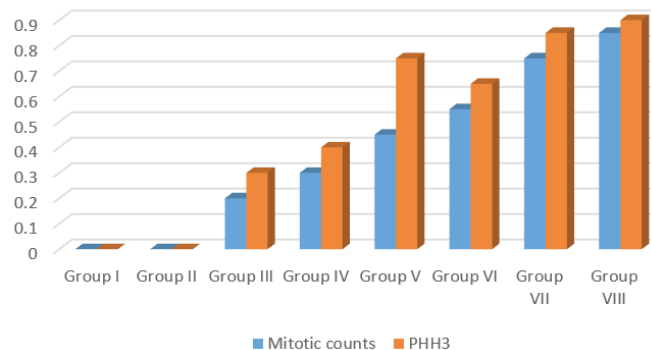


Fig 1. Distribution of mitotic counts and phosphohiston-H3 in different groups, Group I, serous cystadenoma, Group II, mucinous cystadenoma, Group III, serous BOT, Group IV, mucinous BOT, Group V, low grade serous carcinoma, Group VI low grade mucinous carcinoma, Group VII, high grade serous carcinoma, Group VIII, high grade mucinous carcinoma

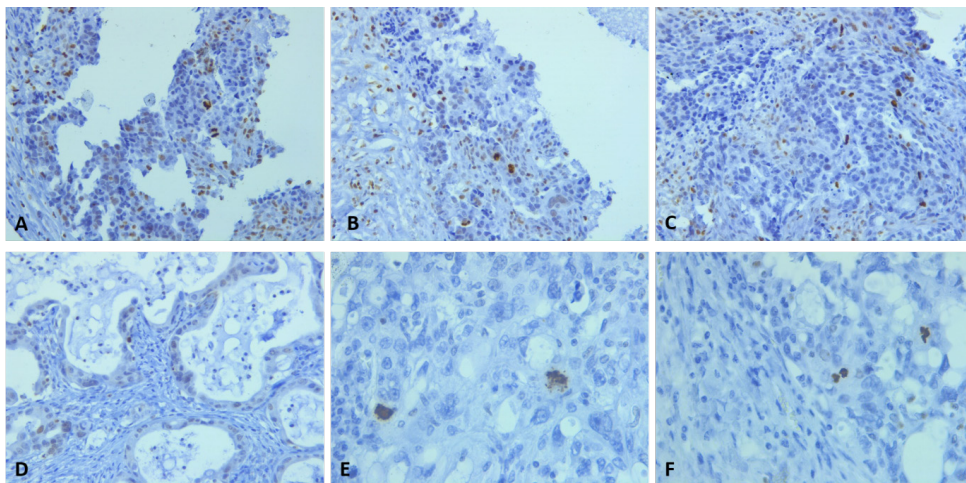


Fig. 2. The expression of PHH3 in A. serous BOT, B. mucinous BOT, C. low grade serous carcinoma, D. low grade mucinous carcinoma, E. high grade serous carcinoma, F. high grade mucinous carcinoma, IHC, X200

Expression of phosphohiston-H3 in different groups with different proliferation potential. Third we analysed the relationship between the expression of PHH3 and Ki67 proliferation marker. We have found high positive correlation ($r=.59$, $p<0.05$) between PHH3 and Ki67 expression. In addition, we have divided groups into additional two groups according to Ki67 proliferation status, particularly patients with low/negative ($\leq 10\%$) Ki67 labelling and patients with high Ki67 ($>10\%$) labelling index. Interestingly we have found, that 30% of cases with low/negative Ki67 labelling index are characterised with high expression of PHH3.

Relationship between phosphohiston-H3 expression and mutant-p53 and BCL2 expression. We have found the moderate positive correlation between phosphohiston-H3 expression and mutant-p53 expression ($r=.42$, $p<0.05$), whilst we have found the moderate negative correlation between phosphohiston-H3 expression and BCL2 expression ($r=.39$, $p<0.05$).

Relationship between phosphohiston-H3 expression and ER and PR. The expression of PHH3 was negatively associated with the expression of ER and PR receptors ($r=.36$, $p<0.05$ and $r=.33$, $p<0.05$ respectively).

First, we counted raw number of mitoses in different groups of ovarian epithelial tumors. The results of our study showed that there are no mitoses detected in benign lesions such as serous and mucinous cystadenomas. With regards to serous and mucinous BOTs, mitotic figures were detected in 20 and 30% of cases respectively. Borderline ovarian tumors (BOTs) are tumors of epithelial origin, which show the intermediate features between benign cystadenomas and adenocarcinomas. In 1973 BOTs were classified by the World Health Organization (WHO) as 'low malignant potential ovarian tumors and in 2003 they were reclassified as 'borderline tumors' [7]. BOTs comprise of approximately 15-20% of all atypical proliferations in the ovary and 4-14% of ovarian epithelial tumours [8]. Even though serous cystadenoma can progress to BOT and finally to serous low-grade carcinoma, these three tumors are recognised as separate entities, which differ in biological behaviour and require different clinical management strategies [8,9]. Most of the BOT cases are at first encountered by general gynaecologists, which leads to inappropriate treatment and delayed management by gynaecologic oncologists. Therefore, early recognition and diagnosis of BOTs are important for the proper management of patients, to avoid disease progression and relapse. Histologically, there are two major types of BOTs: serous-papillary and mucinous. The malignancy potential of both types of BOTs, still requires further investigation. Providing that some of the BOTs in our study show high numbers of mitotic cell counts, we recommend the assessment of mitoses as an additional marker of BOT malignancy potential.

Second, we evaluated the raw number of PHH3 positive cells in different groups of ovarian epithelial tumors. The results of our study showed the high correlation between raw mitotic counts and PHH3 expression ($r=.71$, $p<0.05$). However, the number of PHH3 positive cases were higher compared to cases with the raw mitotic count. Particularly, PHH3 positivity was detected in 30% of serous BOTs, 40% of mucinous BOTs, 75% of low-grade serous carcinomas, 65% of mucinous carcinomas, 85% of high-grade serous carcinomas and 90% of high grade mucinous carcinomas. In addition, we have analysed the relationship between PHH3 and Ki67 expression and we have found that some Ki67 negative cases show the expression of PHH3. These data collectively suggest that the staining with PHH3 is the superior for the detection of mitotic counts and proliferation potential using Ki67.

We have also studied the relationship between PHH3 and mutant p53. The antibody we used against p53 is the cocktail of mutant and normal p53. Therefore, P53 immunohistochemical expression is characterised with three different staining patterns, such as complete absence of staining, wild type positivity and high expression of p53, with different significance. The low or absent expression of p53 indicates the loss of p53 and therefore mutant p53. Wild type expression indicates, which is p53 moderate expression in 60-70% cells indicates the presence of wild type, aka. Normal p53 and the strong dark expression of p53 indicates the presence of mutant p53 [10]. We have found that mutant p53 is negatively associated with the expression of PHH3, meaning PHH3 is increased in more aggressive cancer types. In line with these results, we have also found the negative association of PHH3 with BCL2, ER and PR receptors.

PHH3 has been studied in different types of cancers as a prognostic marker. Nakashima et al, have found that high number of PHH3 positive mitotic cells were associated with poor prognosis in human oesophageal squamous cell carcinoma [11]. Similar to our study, Khieu et al., also found that PHH3 correlates best to histologic grade and has less interobserver variability compared with Ki-67 proliferation index and mitotic cell count in follicular lymphoma cases [12].

Conclusions. To the best of our knowledge it is the first profound study of the phosphorylated histone-H3 in patients with epithelial ovarian tumors. The results of our study show that PHH3 antibodies can be used for the detection of the cell proliferation and the progression potential of the epithelial tumors of the ovary.

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SUMMARY

EPIGENETIC CHANGES – HISTONE 3 PHOSPHORYLATION – EPITHELIAL OVARIAN TUMORS

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Histone modifications represent one of the types of epigenetic changes. Histones, undergo different types of epigenetic modifications, including the phosphorylation of serine residues. pHH3 antibodies specifically detect histon-3 protein, when phosphorylated at 10th and 28th serine residues. Traditionally pHH3 antibodies are used as proliferation marker, as it detects cells in late G2 and M phase. We studied the distribution of phosphor-histon-3 in epithelial tumors of the ovary and its relationship with ER, PR, Ki67, p53 and BCL2. Altogether, we investigated postoperative material from 160 patients. Standard immunohistochemistry was used to detect, phosphohistone-H3 (pHH3), ER, PR, Ki67, p53 and BCL2. The results of our study showed that phosphohistone-H3 expression is negatively associated with the expression of ER and PR expression, as well as with BCL2 expression, on the other hand it positively correlates with Ki67 and mutant p53 ($p < 0.05$). In addition, the expression of phosphohistone-H3 is detected in Ki67 negative cases and its expression is increased along with the increase of malignancy grade. Our study results indicate that PHH3 might be used as an additional marker for the assessment of proliferation and malignancy potential of epithelial tumors of the ovary.

Keywords: phosphohistone-H3, epithelial tumors of the ovary.

РЕЗЮМЕ

ОСОБЕННОСТИ ЭПИГЕНЕТИЧЕСКИХ ИЗМЕНЕНИЙ – ФОСФОРИЛИРОВАНИЕ ГИСТОНА-3, В ЭПИТЕЛИАЛЬНЫХ ОПУХОЛЯХ ЯИЧНИКА

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Модификация гистонов - один из видов эпигенетических изменений. Гистоны подвергаются различным эпигенетическим модификациям, в том числе фосфорилированию сериновых остатков. Антитела pHH3 специфически выявляют белок гистон-3 в случае его фосфорилирования в 10 и 28 сериновых остатках. Традиционно его используют как мар-

кер клеточной пролиферации и выявляют клетки в поздних G2 и M фазах. Целью исследования явилось определение особенности распределения фосфолированного гистона-3 в эпителиальных опухолях яичника и его связи с маркерами ER, PR, Ki67, p53, BCL2 и WT1. Изучены постоперационные материалы 160 пациентов. Проведено стандартное иммуногистохимическое исследование с использованием маркеров: фосфо-гистон-3, ER, PR, Ki67, p53, BCL2 и WT1.

Результаты исследования выявили, что экспрессия фосфо-гистона-3 находится в негативной ассоциации с экспрессией ER, PR рецепторов и BCL2, в позитивной корреляции с Ki67 и наличием мутантного p53 ($p < 0.05$). Фосфо-гистон-3 отмечается и при Ki67-негативных случаях и его содержание увеличивается с ростом степени злокачественности. Результаты исследования показали, что фосфолированный гистон-3 используется в качестве дополнительного маркера определения пролиферативного и злокачественного потенциала эпителиальных опухолей яичника.

რეზიუმე

ეპიგენეტიკური ცვლილებების თავისებურებები – ჰისტონ-3-ის ფოსფორილება, საკვრცხის ეპითელურ სიმსივნეებში

ვ.მუნჯიშვილი, ე.ბარაბაძე, თ.მუზაშვილი, მ.გაჩეჩილაძე, გ.ბურკაძე

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ჰისტონების მოდიფიკაცია წარმოადგენს ეპიგენეტიკური ცვლილებების ერთ-ერთ სახეობას. ჰისტონები ექვემდებარება სხვადასხვაგვარ ეპიგენეტიკურ მოდიფიკაციებს, რომელთა შორის არის სერინული ნაშთების ფოსფორილება. pHH3 ანტისხეულები სპეციფიკურად ავლენს ჰისტონ-3 ცილას, როდესაც იგი ფოსფორილებულია სერინის მე-10 და 28-ე ნაშთებში. ტრადიციულად იგი გამოიყენება, როგორც უჯრედული პროლიფერაციის მარკერი და ავლენს უჯრედებს გვიან G2 და M ფაზებში.

კვლევის მიზანს წარმოადგენდა ფოსფორილებული ჰისტონ-3-ის განაწილების თავისებურების შესწავლა საკვრცხის ეპითელურ სიმსივნეებში და მისი კავშირის დადგენა ისეთ მარკერებთან, როგორცაა ER, PR, Ki67, p53 BCL2 და WT1.

გამოკვლეულია 160 პაციენტის პოსტოპერაციული მასალა. სტანდარტული იმუნოჰისტოქიმიური მეთოდით შესწავლილია ფოსფო-ჰისტონ-3, ER, PR, Ki67, p53, BCL2 და WT1. კვლევის შედეგებმა გამოავლინა, რომ ფოსფო-ჰისტონ-3-ის ექსპრესია ნეგატიურ ასოციაციაშია ER, PR რეცეპტორების ექსპრესიასთან და BCL2-თან. პოზიტიურ კორელაციაშია Ki67 და მუტანტ p53-თან ($p < 0.05$). ფოსფო-ჰისტონ-3 აღინიშნება Ki67 ნეგატიურ შემთხვევებშიც და მისი მოცულობა იზრდება ავთვისებიანობის ხარისხის ზრდასთან ერთად.

ავტორების მიერ გამოტანილია დასკვნა, რომ ფოსფორილებული ჰისტონ-3 შესაძლებელია გამოყენებული იყოს, როგორც საკვრცხის ეპითელური სიმსივნეების პროლიფერაციული და ავთვისებიანობის პოტენციალის განსაზღვრის დამატებითი მარკერი.

ABILITY TO FORM BIOFILMS BY PYELONEPHRITIS CAUSATIVE AGENTS IN CHILDREN

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The course of infectious diseases can proceed with complications just because of the formation of microbial biofilms in organism. Many chronic diseases, such as urinary tract infections including pyelonephritis, are associated with biofilm infections. [1,2]. Planktonic bacteria are known to reach the kidneys in an ascending way and be able to attach to uroepithelium and kidney papillae in kidney collective systems. Bacterial adhesion is the main moment in tissue surface of host organism. Adhesion of microorganisms to uroepithelium enables them to resist removal by urine flow. Bacterial adhesion not only promotes colonization, but also favors invasion of microorganisms, biofilms formation and damage of host cells with pyelonephritis development. Biofilms on uroepithelium surface are easy eradicated by antimicrobial agents compared with biofilms formed on alien objects in urinary system. Alien body in urinary tract (catheters, stents, drainages, stones) becomes the infection source for the organism which leads to the development of urinary system complicated infections [3,4]. Periodic release of bacteria planktonic forms from biofilms with urine flow is the source of maintenance of chronic infections and inflammatory process in kidneys.

The biofilms study nowadays attracts great interest of the researches mainly for the reason that this way of bacteria existence creates more problems in medical practice. Biofilms present one of pathogenic factors of pyelonephritis chronic forms formation [5, 6].

Bacteria living in biofilms significantly differ from planktonic forms in their biological properties. Bacteria stability in biofilms to antibiotics and attack of immune system draws more attention. The nature of this stability is intensely studied at present. Thus, the ability of biofilms bacteria to survive in presence of antibiotics in concentrations sufficiently exceeding standard therapeutic concentrations creates difficulties in pyelonephritic treatment in children as this poses production of polyresistant planktonic cells the result of which is the appearance of chronic pyelonephritis and frequent relapses [7].

The problem of suppression and destruction of bacterial biofilms is the extremely urgent task as in clinics classical methods of antibiotic therapy of purulent-inflammatory infections are of-

ten ineffective and unpredictable because of high resistance of causative agents in biofilms.

Thus, at present means and methods allowing the destruction of biofilms and facilitating access of antimicrobial preparations to planktonic cells are actively sought. Thorough study of biofilms formation processes by pyelonephritis causative agents with the help of light, fluorescent and scanning microscopy is necessary [8].

The aim of the given study was the detection of the ability to biofilms formation by bacteria, pyelonephritis causative agents in children with the help of light, fluorescent and scanning microscopy.

Material and methods. Bacteriological method for microorganism identification according to accepted microbiological schemes of microorganisms allocation and identification was used to achieve the goal [9]. Sterile polymeric Petri dishes d=40 mm were used to receive biofilms. Each dish was placed 4 ml of Müller-Hinton broth and daily microorganisms culture excreted from urine in children with pyelonephritis and incubated for 12-24 hours under +37°C. After incubation the growth media was poured out, the dishes were rinsed twice by Hencs solution (2 ml), fixed by 10% solution of formalin on distilled water (pH=7,2-7,4), dried, stained by 1% solution of crystal violet and washed by distilled water [10].

The preparations microscopy was done with the help of Gram microscope with oil immersion. Bacteria and their biofilms digital imaging was received with the help of TouPCam 3.1 MP video camera (video ocular) and saved in jpeg format.

Scanning (the department of experimental physics of the physical faculty of V.N.Karazin KNU) and fluorescent microscopy (for daily biofilms staining 200 mcl of working mixture of acridine orange (concentration 2 mcg/ml) were used for visualization of biofilms morphologic structure in high resolution. The records were kept with the help of fluorescent microscope, enlargement 100x10x1,5, using filters, providing exciting light with the wave length not more than 490 nm and emission with the wave length 520 nm).



Fig. 1. Determination of biofilm formation ability with the help of light, fluorescent and scanning microscopy (I – light microscope; II – fluorescent and scanning microscope)

Results and their discussion. The study demonstrated that all isolates formed biofilms. Adhesion of planktonic bacteria forms took place on the first stage, intracellular matrix formation occurred on the second stage and biofilm formation took place on the third

stage. During the study of *E. coli* and *Proteus spp.* bacteria preparations with the use of scanning and light microscopy ordered bacteria arrangement was seen in the form of separate structures or tiny clusters of bacterial cells united by matrix (Fig. 2, 3).

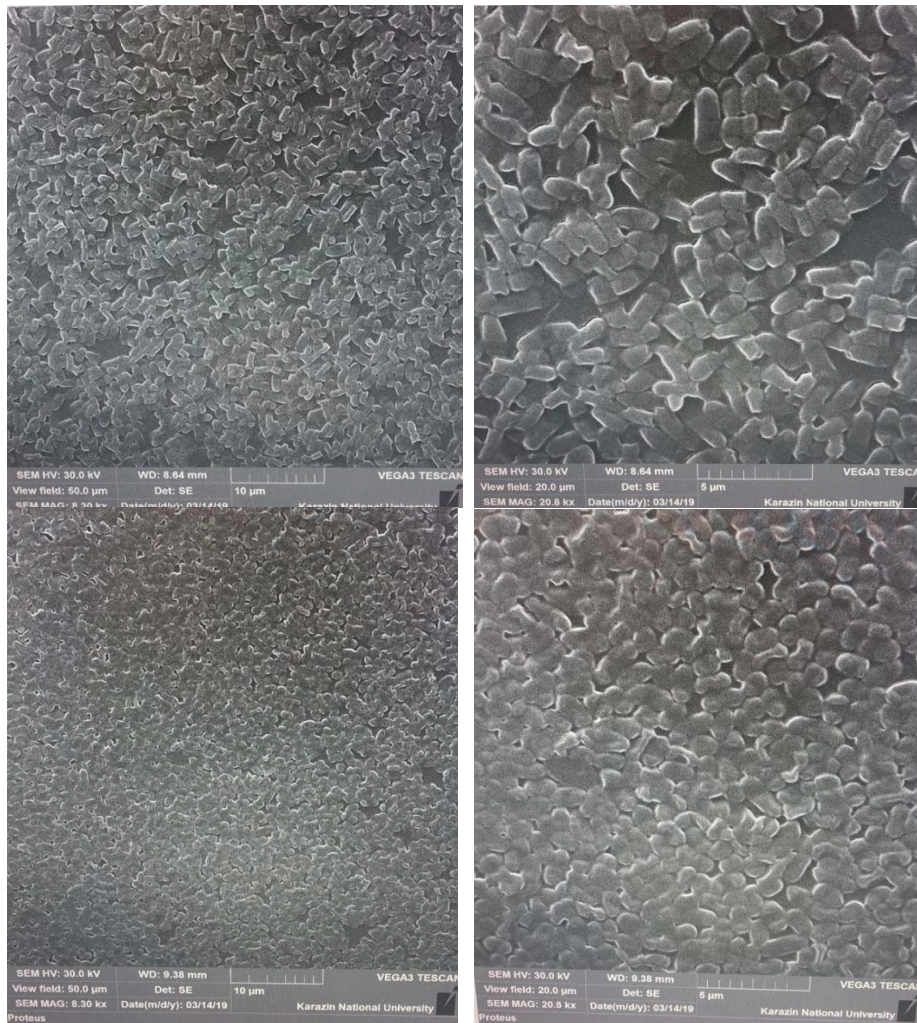


Fig. 2. Bacteria biofilms formation: 1 – *E. coli*; 2 – *Proteus spp.*; scanning microscopy

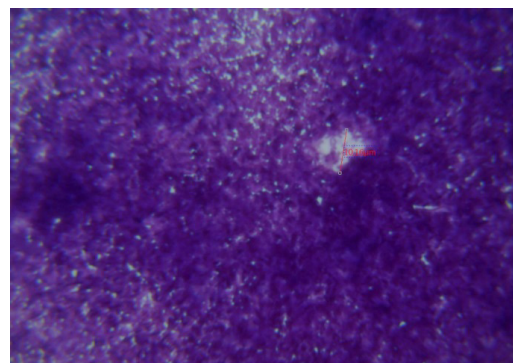
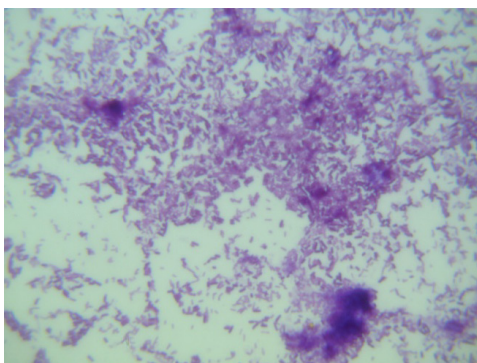


Fig. 3. *E. coli* isolates biofilms formation: 1 – with acute form of pyelonephritis; 2 – with chronic form of pyelonephritis. Light microscopy

During the study of the ability to form *P. aeruginosa* isolates biofilms with the help of scanning microscopy it was stated that the adhesion of separate bacterial cells occurs with further conglomerates formation which are surrounded

by matrix with further biofilm formation (Fig. 4). Packed biofilms areas with cells clusters with good fluorescence were found with the help of fluorescent microscopy (Fig. 5).

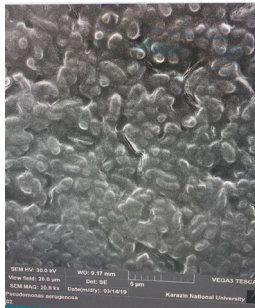


Fig. 4. *P. aeruginosa* bacteria biofilms formation.; scanning microscopy

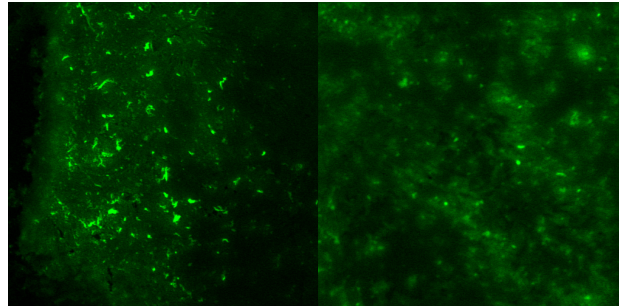


Fig. 5. Ability to *P. aeruginosa* bacteria biofilms formation.; fluorescent microscopy

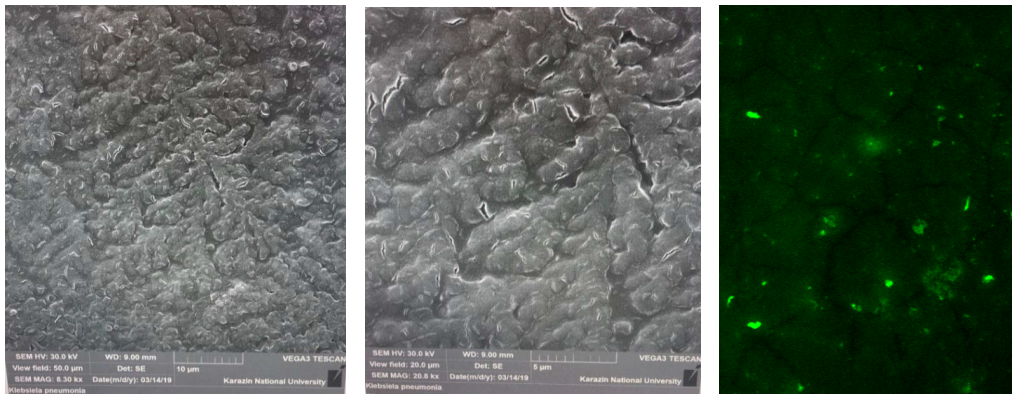


Fig. 6. *K.pneumoniae* bacteria biofilms formation; scanning and fluorescent microscopy

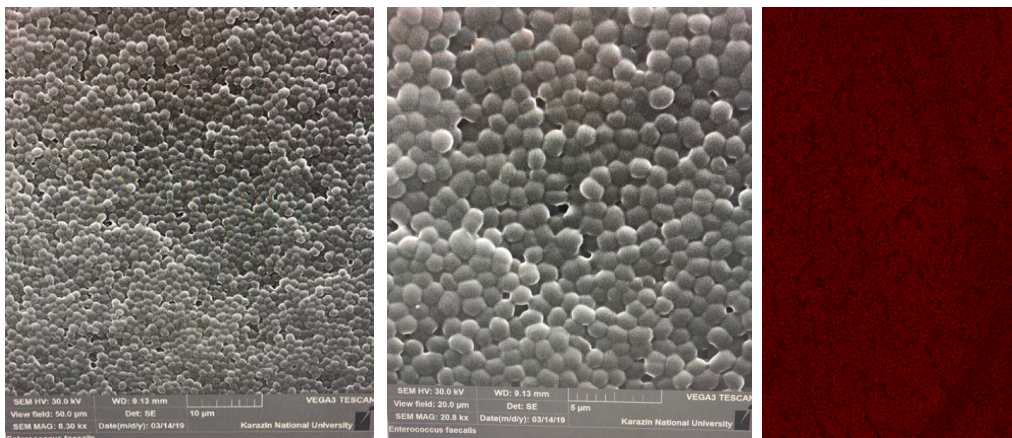


Fig. 7. *E. Faecalis* bacteria biofilms formation; scanning and fluorescent microscopy

During daily *K.pneumoniae* isolates biofilms study by methods of scanning and fluorescent microscopy (Fig. 6) it was found by that *K.pneumoniae* biofilms were covered with dense matrix and riddled with multiple canals in the form of apertures.

During morphological peculiarities study of *E. faecalis* isolates biofilms formation with the use of scanning and fluorescent microscopy it was found that bacterial cells were densely packed and united by intracellular matrix under which bacteria of spherical shape were seen. (Fig. 7).

Thus biofilms, the nature of which depends on the type of bacteria, are formed on the surface of conglomerates consisting of bacterial cells. Peculiarities of course and appearance of pyelonephritis chronic form and relapses in children is explained by biofilms formation.

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SUMMARY

ABILITY TO FORM BIOFILMS BY PYELONEPHRITIS CAUSATIVE AGENTS IN CHILDREN

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The work is dedicated to the study of biofilms formation process by main pyelonephritis causative agents in children *in vitro* using methods of light, fluorescent and scanning microscopy. To study biofilms formation bacteria were cultivated in liquid substratum on glass in polystyrene Petri dishes d=40mm. The study demonstrated that all isolates formed biofilms. Adhesion of bacteria planktonic forms took place on the first stage, intracellular matrix formation took place on the second stage, and biofilms formation took place on the third stage.

During the study of *E. coli* and *Proteus spp* bacteria preparations with the use of scanning and light microscopy ordered bacteria arrangement was seen in the form of separate structures or tiny clusters of bacterial cells united by matrix. During the study of the ability to form *P. aeruginosa* isolates biofilms with the help of scanning microscopy it was stated that the adhesion of separate bacterial cells occurs by conglomerates formation surrounded by matrix with further

biofilms formation. Bacterial cells in the form of dense elongated sticks were seen under the film. *P. aeruginosa* isolates daily biofilms were stated to have dense structure in the form of gel. Packed biofilms areas with cells clusters with good fluorescence were found with the help of fluorescent microscopy. During daily *K.pneumoniae* isolates biofilms study by methods of scanning and fluorescent microscopy it was found that *K.pneumoniae* biofilms were covered with dense matrix and riddled with multiple canals in the form of apertures. During morphological peculiarities study of *E. faecalis* isolates biofilms formation with the use of scanning and fluorescent microscopy it was found that bacterial cells were densely packed and united by intracellular matrix under which bacteria of spherical shape were seen.

Thus biofilms, the nature of which depends on the type of bacteria, are formed on the surface of conglomerates consisting of bacterial cells. Peculiarities of course and appearance of pyelonephritis chronic form and relapses in children is explained by biofilms formation.

Keywords: bacteria biofilms, isolates, scanning, light, fluorescent microscopy, pyelonephritis in children.

РЕЗЮМЕ

СПОСОБНОСТЬ К ОБРАЗОВАНИЮ БИОПЛЕНОК ВОЗБУДИТЕЛЯМИ ПИЕЛОНЕФРИТОВ У ДЕТЕЙ

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Статья посвящена исследованию процесса формирования биопленок основными возбудителями пиелонефритов у детей *in vitro* с использованием методов световой, люминесцентной и сканирующей микроскопии. В результате исследования установлено, что все изоляты образовывали биопленки.

Выявлено упорядоченное расположение изолятов *E. coli* и *Proteus spp*. в виде отдельных структур или небольшого скопления бактериальных клеток, объединенных матриксом. Установлено, что адгезия отдельных бактериальных клеток *P. aeruginosa* происходит с формированием конгломератов, окруженных матриксом с последующим образованием биопленки.

Выявлено, что суточные биопленки изолятов *P. aeruginosa* имеют плотную структуру в виде геля, биопленки *K.pneumoniae* покрыты плотным матриксом и пронизаны множественными каналами в виде отверстий, изоляты *E. faecalis* плотно упакованы и объединены межклеточным матриксом, под которым видны бактерии шаровидной формы.

Таким образом, на поверхности конгломератов, состоящих из бактериальных клеток, формируются биопленки, природа которых зависит от вида бактерий. Формированием биопленок объясняются особенности течения и возникновения хронической формы и рецидивов пиелонефрита у детей.

რეზიუმე

ბავშვებში პიელონეფრიტის გამომწვევი აგენტების მიერ ბიოაპკების ფორმირების უნარი

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ხარკოვის ეროვნული სამედიცინო უნივერსიტეტი, ¹დ. გრინვეის სახ. მიკრობიოლოგიის, ვირუსოლოგიისა და იმუნოლოგიის კათედრა; ²პედიატრიის №2 კათედრა; ³ხარკოვის ვ.კარაზინის სახ. ეროვნული უნივერსიტეტი, კლინიური იმუნოლოგიისა და ალერგოლოგიის კათედრა, უკრაინა

ნაშრომი ეძღვნება პიელონეფრიტის ძირითადი გამომწვევების მიერ ბიოაპკების წარმოქმნის პროცესის *in vitro* კვლევას ბავშვებში სინათლის, ლუმინესცენტური და მასკანირებელი მიკროსკოპიის მეთოდების გამოყენებით. კვლევის შედეგად დადგენილია, რომ ბიოაპკებს წარმოქმნის ყველა იზოლატი. გამოვლენილია *E. coli* და *Proteus spp.*-ის იზოლატების თანმიმ-

დევრული განაწილება ცალკეული სტრუქტურების ან მატრიქსით გაერთიანებული ბაქტერიული უჯრედების მცირე დაჯგუფებების სახით. დადგენილია, რომ *P. aeruginosa*-ას ცალკეული ბაქტერიული უჯრედის ადჰეზია ხორციელდება მატრიქსით გარშემორტყმული კონგლომერატების ფორმირებით, ბიოაპკის შემდგომი წარმოქმნით. გამოვლენილია, რომ *P. aeruginosa*-ას იზოლატების დღედაღამურ ბიოაპკებს აქვს მკვრივი გელისმაგავრი სტრუქტურა, *K.pneumoniae* -ს ბიოაპკები დაფარულია მკვრივი მატრიქსით და გამსჭვალულია ნახერცების სახით მრავალრიცხოვანი არხებით, *E. Faecalis*-ის იზოლატები მკვრივად შეკრული და გაერთიანებული უჯრედშორისი მატრიქსით, რომლის ქვეშაც აღინიშნება სფეროს ფორმის ბაქტერიები.

ამრიგად, ბაქტერიული უჯრედებისაგან შემდგარი კონგლომერატების ზედაპირზე ფორმირდება ბიოაპკები, რომელთა თვისებები ბაქტერიების სახეობაზე დამოკიდებულია. ბიოაპკების ფორმირებით აიხსნება პიელონეფრიტის მიმდინარეობის, ქრონიკული ფორმების და რეციდივების განვითარების თავისებურებები ბავშვებში.

ДИНАМИКА ИЗМЕНЕНИЙ УЛЬТРАСТРУКТУРЫ МАКРОФАГОЦИТОВ РАНЕВОГО КАНАЛА ПОСЛЕ ОГНЕСТРЕЛЬНОГО РАНЕНИЯ

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Несмотря на стремительное развитие современных средств поражения во время локальных военных конфликтов, миротворческих миссий и военных операций, наибольшее количество повреждений наносится огнестрельным оружием [4,17,19].

В структуре огнестрельных ранений, полученных во время вооруженных конфликтов, преобладают травмы конечностей с повреждением костей и мягких тканей. По результатам анализа случаев ранений американских военнослужащих во время второй мировой, вьетнамской, иракской войны, доля проникающих ранений конечностей составляет от 50% до 70%, среди них только у трети пострадавших были пулевые ранения [2,5,13].

По данным J.J. Doucet и соавт. [8], боевая травма, в отличие от гражданской, в большинстве случаев (63,3%) повреждает нижние конечности, в основном, в результате взрывов, в 16,3% - от высокоскоростных огнестрельных ранений.

Несмотря на многовековую историю применения огнестрельного оружия, углубление данных патогенеза огнестрельных ран и множества предложенных методик оказания медицинской помощи [10,11,16,18], вопросы длительности восстановления мягких тканей после огнестрельных осколочных ранений по сей день не изучены [1,15].

Недостаточно изучен вопрос длительности изменений и

полноценности восстановления макрофагальной системы рубцовых тканей после огнестрельных осколочных ранений. Одним из методов, позволяющих оценить восстановление функционирования и структуры тканей на субклеточном уровне является электронная микроскопия [3,6,14].

Цель исследования - определить особенности перестройки субмикроскопической архитектоники макрофагоцитов скелетных мышц, динамику трансформаций органелл и внутриклеточных мембран в различные сроки после моделированного огнестрельного осколочного ранения.

Материал и методы. Для реализации поставленной цели исследования использованы 26 лабораторных животных – племенных кроликов породы «Шиншилла», с массой тела 2200-3000 гр. Средняя масса животных составила 2620±120 гр. За 20 минут до начала экспериментов проводили обезболивание лабораторных животных препаратом налбуфин в дозе 0,3 мг/кг массы тела животного, внутримышечно. Лабораторным животным наносили огнестрельное ранение в области мышц бедра из пистолета «Форт-12» калибр 9 мм, с усиленным патроном, заряженным обрезанными (без шляпки) металлическими шурупами СМК 3,5x9,5 («саморез») массой 0,9-1,1 гр с дистанции 3,0 м. Начальная скорость осколка составила 305 м/сек. Моделирование огнестрельных осколочных ранений проводилось в сертифици-

цированном стрелковом тире с соблюдением требований безопасности. Все огнестрельные ранения были слепыми, осколки оставались в мягких тканях животных, раны заживали с осколками. Кролики выводились из эксперимента на 30 и 60 сутки после нанесения ранений.

Методом случайных чисел кролики были рандомизированы на следующие группы:

I группа (контрольная) – 6 интактных кролей, которым огнестрельные ранения не наносились, из эксперимента выводились по 3 кроля на 30 и 60 сутки, материал из мышцы бедра забирали для контроля качества подготовки препаратов для электронной микроскопии.

II группа – 10 кролей, которым ранения наносились по описанной выше методике, животные выводились из эксперимента на 30 сутки после огнестрельного ранения.

III группа – 10 кролей, ранения которым наносились также по описанной выше методике, животные выводились из эксперимента на 60 сутки после огнестрельного ранения.

Лабораторных животных содержали в виварии Харьковской медицинской академии последипломного образования в одинаковых стандартизированных условиях с использованием естественного светового режима и стандартной диеты, при свободном доступе к воде и пище, согласно международным правилам «Руководства по уходу и использованию лабораторных животных» [12]. Экспериментальные работы проводились согласно европейским требованиям обращения с животными [7,9].

Протоколы исследований с использованием лабораторных животных одобрены локальной этической комиссией Военно-медицинского клинического Центра Северного региона (положительное решение Комиссии по вопросам этики №3/2 от 12.03.2015 г. «О проведении исследований огнестрельных ранений мягких тканей с использованием лабораторных животных»).

Кусочки рубцовой ткани, забранные из раневого канала, иссекали для электронно-микроскопического исследования, помещали для предварительной фиксации в 2,5% забуференный раствор глутарового альдегида на 5-6 часов при температуре 4°C. После окончания предварительной фиксации кусочки ткани промывали в буферном растворе и переносили в 1% забуференный раствор четырехоксида осмия на 2-3 часа при температуре 4°C. Ткань обезвоживали в спиртах возрастающей концентрации и ацетоне, пропитывали смесью эпоксидных смол (эпо-аралдит) и заключали в блоки по общепринятой методике. Полимеризацию блоков проводили в термостате при температуре 60°C в течение двух суток.

Из полученных блоков на ультрамикротоме УМП-4 (Сумский завод электронных микроскопов, Украина) изготавливали ультратонкие срезы, которые после контрастирования цитратом свинца изучали под электронным микроскопом ЭМ-125 (Сумский завод электронных микроскопов, Украина) при ускоряющем напряжении 75 кВ.

Контролем качества гистологической обработки ткани служили биоптаты интактных экспериментальных животных. Электронное микроскопическое исследование ультраструктурной организации миосимпластов скелетных мышц интактных экспериментальных животных показало адекватность гистологической обработки тканей. Субмикроскопические структуры миосимпластов соответствовали современным представлениям. Мембраны, которые образовывали органеллы имели четкую контурированную структуру, свойственную элементарной мембране, без очагов деструкции.

Результаты и их обсуждение. На тридцатые сутки после огнестрельного ранения в рубцовой ткани, окружающей раневой канал, в скелетных мышцах обнаруживались макрофагоциты, субмикроскопическая архитектура которых отличалась полиморфизмом.

В макрофагоцитах частично визуализировались хорошо развитые органеллы, соответствующие типичной ультраструктурной организации этих клеток. Ядра имели неправильную форму, матрикс ядра содержал конденсированный хроматин, который в виде осмиофильных глыбок локализовался на ядерной мембране. Гранулы деконденсированного хроматина и рибосомы рассеяны в центральной области матрикса ядра. Ядерная мембрана четко контурирована, без очагов разрушения и разрыхления. Наблюдаются глубокие инвагинации ядерной мембраны. Очаги деструкции кариолеммы и локальные расширения перинуклеарных пространств отсутствовали. Гранулярный эндоплазматический ретикулум представлен умеренно расширенными цистернами, заполненными веществом низкой электронной плотности. На мембранах гранулярного эндоплазматического ретикулума выявляются многочисленные рибосомы. В цитоплазме макрофагоцитов присутствуют многочисленные скопления рибосом и полисом (рис. 1а).

Мелкие митохондрии с небольшим количеством укороченных и разрыхленных крист содержат мелко гранулярный матрикс средней электронной плотности. Пластинчатый цитоплазматический комплекс Гольджи умеренно гипертрофирован, его гладкие мембраны, собранные в пакеты, параллельно ориентированы; пакеты мембран пластинчатого цитоплазматического комплекса Гольджи окружены большим количеством мелких везикул. В цитоплазме макрофагоцитов располагаются первичные лизосомы и включения фагоцитированного материала, по всей вероятности, липопротеидной природы (рис. 1б). Цитоплазматическая мембрана макрофагоцитов имеет типичную для этих клеток структуру, присущую элементарной мембране и не содержит участков, подверженных лизису.

В цитоплазме отдельных макрофагоцитов из раневого канала бедренной мышцы на 30 сутки после огнестрельного ранения обнаруживались макрофагоциты, ультраструктурная организация которых сохраняла деструктивные и дистрофические нарушения органелл (рис. 1в). Ядра макрофагоцитов имеют неправильную форму и содержат большое количество конденсированного хроматина, глыбки которого располагаются как по периферии ядра, так и в других отделах матрикса. Центральная область матрикса ядра имеет низкую электронную плотность и заполнена большим количеством гранул деконденсированного хроматина. В цитоплазме обнаруживаются набухшие митохондрии с электронно-прозрачным матриксом, разрушенными кристами и очагами деструкции наружных мембран. Гранулярный эндоплазматический ретикулум представлен большим количеством мелких электронно-прозрачных цистерн. Кроме того, в цитоплазме присутствуют крупные аутофагосомы.

В препаратах встречаются макрофагоциты, заполненные скоплениями включений липидов и осмиофильного фагоцитированного вещества (рис. 1г).

Субмикроскопическая организация органелл макрофагоцитов из рубцовой ткани раневого канала в скелетных мышцах на 60 сутки после огнестрельного ранения содержит элементы как дистрофических, так и деструктивных нарушений.

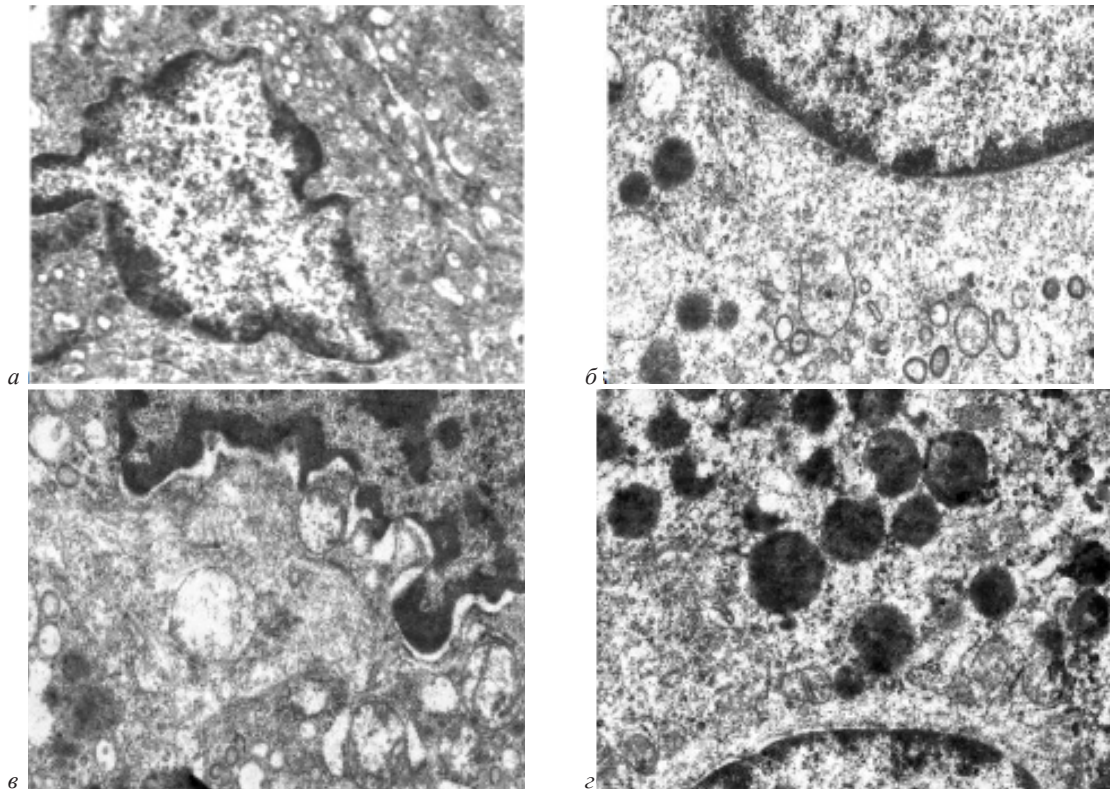


Рис. 1. Ультраструктура макрофагов рубцовой ткани, окружающей раневой канал в скелетных мышцах, 30 суток после огнестрельного ранения. Контрастировано цитратом свинца: а - расширение цистерн гранулярного эндоплазматического ретикулума, X45000, б - вторичные лизосомы и фагоцитированный материал в цитоплазме, X61000, в - набухание митохондрий и очаговая деструкция наружных мембран, X50000, г - скопления осмиофильного фагоцитированного материала в цитоплазме, X46000

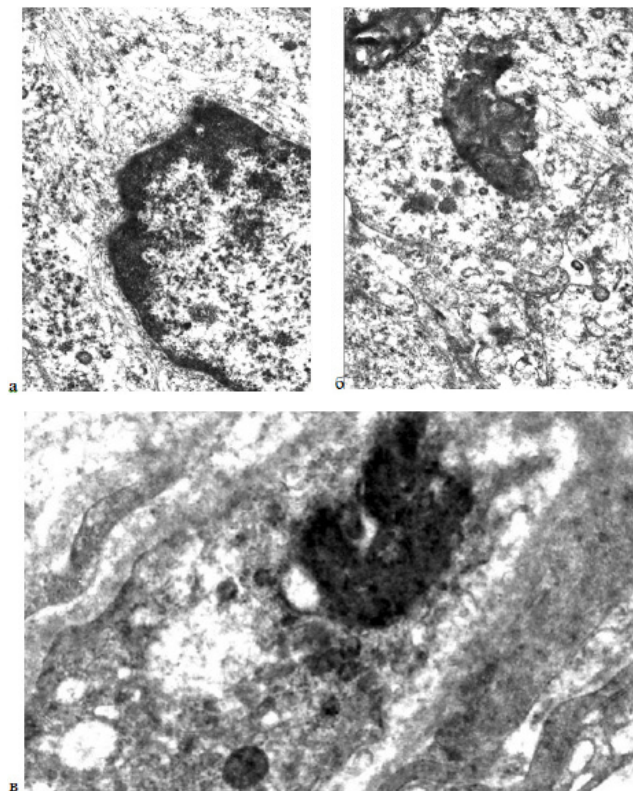


Рис. 2. Ультраструктура макрофагов из рубцовой ткани, окружающей раневой канал в скелетных мышцах, 60 суток после огнестрельного ранения. Контрастировано цитратом свинца: а - конденсированный хроматин ядра, X42000, б - разрушенные митохондрии в цитоплазме, X66000, в - вторичные лизосомы в цитоплазме, X72000

Значительная часть макрофагов отличается запустеванием цитоплазмы. В перинуклеарной области цитоплазмы практически отсутствуют органеллы. Эта область цитоплазмы имеет электронно-прозрачный вид и заполнена небольшим количеством полисом и рибосом. Ядра макрофагов сохраняют неправильную форму. Ядерная мембрана очагово разрушена и местами разрушена. Наблюдаются многочисленные инвагинации оболочки ядра. Перинуклеарные пространства не расширены и заполнены веществом очень низкой электронной плотности. Глыбки конденсированного хроматина ядра концентрируются как на ядерной мембране, так и диффузно рассеяны по матриксу ядра. Центральная область матрикса ядра имеет низкую электронную плотность (рис. 2а).

Цистерны гранулярного эндоплазматического ретикула сильно расширены и электронно-прозрачны. Митохондрии имеют различные размеры и форму, матрикс их варьирован в зависимости от плотности. Кристы митохондрий дезорганизованы и укорочены. У значительной части митохондрий наблюдаются очагово-разрушенные наружные мембраны и кристы (рис. 2б).

В препаратах встречаются отдельные макрофаги, в цитоплазме которых обнаруживаются крупные вторичные лизосомы (рис. 2в).

Заключение. На тридцатые сутки после огнестрельного ранения бедренной мышцы при электронно-микроскопическом исследовании органелл макрофагов, взятых из области рубцовой ткани раневого канала, выявлены полиморфные изменения ультраструктурной архитектоники органелл. Большое количество макрофагов находится в состоянии высокой функциональной активности, о чём свидетельствует хорошо развитый гранулярный эндоплазматический ретикулум, на мембранах которого обнаруживаются многочисленные рибосомы и гипертрофированный пластинчатый цитоплазматический комплекс Гольджи.

В препаратах встречается небольшое количество макрофагов, содержащих органеллы, с выраженными дистрофическими и деструктивными очаговыми разрушениями мембран ядра, митохондрий и гранулярного эндоплазматического ретикула, что свидетельствует об активном течении катаболических процессов, вызванных наличием в раневом канале токсических компонентов, разрушенных миосимпласов и возможным внесением в раневую канал ионов тяжелых металлов из поражающего элемента, что косвенно подтверждается присутствием в цитоплазме вторичных лизосом, включений липидов и большого числа фагоцитированной субстанции.

Электронно-микроскопическое исследование ультраструктурной организации органелл макрофагов из раневого канала на шестидесятые сутки после огнестрельного ранения выявило аналогичные нарушения, имеющие место в предыдущем наблюдении. Необходимо отметить возрастание количества макрофагов, содержащих деструкции мембран органелл, свидетельствующее о возрастании уровня активности катаболических внутриклеточных процессов, которые, по всей вероятности, связаны с прогрессирующим развитием митохондриальной дисфункции и чрезмерным накоплением в цитоплазме макрофагов фагоцитированного вещества.

Высокая активность макрофагов, на наш взгляд, обусловлена вяло текущими процессами утилизации разрушенных ультраструктурных компонентов миосимпласов. Не исключена и возможность токсического влияния ионов

тяжелых металлов из поражающего элемента, что подтверждается присутствием в препаратах макрофагов, цитоплазма которых заполнена большим количеством фагоцитированного материала и органелл, подверженных деструктивным изменениям. Вышеизложенное указывает на высокий уровень катаболических реакций.

Выявленные изменения ультраструктурной организации органелл макрофагов показали, что в области раневого канала сохраняется высокая активность макрофагов, связанная с вяло текущим воспалительным процессом.

Выводы. 1. Электронно-микроскопическое исследование органелл макрофагов, забранных из области рубцовой ткани раневого канала на тридцатые сутки после огнестрельного ранения бедренной мышцы, выявило полиморфные изменения их ультраструктурной архитектоники.

2. Большое количество макрофагов находилось в состоянии высокой функциональной активности, о чём свидетельствует хорошо развитый гранулярный эндоплазматический ретикулум, на мембранах которого обнаруживались многочисленные рибосомы, а также гипертрофированный пластинчатый цитоплазматический комплекс Гольджи.

3. Выраженные дистрофические и деструктивные изменения органелл с очаговыми разрушениями мембран ядра, митохондрий и гранулярного эндоплазматического ретикула в препаратах обнаруживались редко.

4. Интенсивность катаболических процессов обусловлена наличием в раневом канале токсических компонентов, разрушенных миосимпласов, а также возможным внесением в раневую канал ионов тяжелых металлов из поражающего элемента.

5. На шестидесятые сутки после огнестрельного ранения высокая активность макрофагов обусловлена вяло текущими процессами утилизации разрушенных ультраструктурных компонентов миосимпласов и ионов тяжелых металлов из поражающего элемента и вяло текущим воспалительным процессом.

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SUMMARY

DYNAMICS OF CHANGES IN THE MACROSTRUCTURE OF MACROPHAGE CELLS OF THE WOUND CHANNEL AFTER A GUNSHOT WOUND

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The purpose of this study was to identify the features of skeletal muscle macrophagocytic submicroscopic architectonics, the dynamics of transformations of organelles and intracellular membranes at different time after the simulated gunshot wound.

The study involved 26 breeding rabbits. Gunshot wounds were inflicted from a Fort 12 pistol. Rabbits were removed from the experiment on the 30th and 60th day, after the application of wounds. Ultrathin sections, after contrasting with lead citrate,

were examined under an EM-125 electron microscope at an accelerating voltage of 75 kV, with an increase of 42,000–72,000.

Electronic microscopic studies of macrophagocyte organelles taken from the scar tissue of the wound channel on the thirtieth day after a gunshot wound revealed a large number of macrophage cells that are in a state of high functional activity, dystrophic and destructive changes of organelles with focal disruption of the membrane of the nucleus, mitochondria and the granular endoplasmatic reticulum. The course of catabolic processes caused by the presence in the wound channel of toxic components, destroyed myosimplasts and heavy metal ions of the damaging element. On the sixtieth day after a gunshot wound, the increased activity of macrophage cells remained, due to sluggish current processes of utilization of the destroyed ultrastructural components of myosimplasts and heavy metal ions from the damaging element.

Keywords: macrophagocyte ultrastructure, gunshot wound, dystrophic changes of organelles.

РЕЗЮМЕ

ДИНАМИКА ИЗМЕНЕНИЙ УЛЬТРАСТРУКТУРЫ МАКРОФАГОЦИТОВ РАНЕВОГО КАНАЛА ПОСЛЕ ОГНЕСТРЕЛЬНОГО РАНЕНИЯ

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Целью данного исследования явилось определение особенностей перестройки субмикроскопической архитектоники макрофагоцитов скелетных мышц, динамики трансформаций оргanelл и внутриклеточных мембран в различные сроки после моделированного огнестрельного осколочного ранения.

Наблюдались 26 племенных кроликов, огнестрельные ранения которым наносились из пистолета «Форт –12». Кролики выводились из эксперимента на 30 и 60 сутки после нанесения ранения. Ультратонкие срезы, после контрастирования цитратом свинца, изучали под электронным микроскопом ЭМ-125 (Сумский завод электронных микроскопов, Украина) при ускоряющем напряжении 75 кВ, с увеличением 42000–72000.

Электронно-микроскопические исследования оргanelл макрофагоцитов, забранных из рубцовой ткани раневого канала на тридцатые сутки после огнестрельного ранения, выявили большое количество макрофагоцитов, находящихся в состоянии высокой функциональной активности, дистрофические и деструктивные изменения оргanelл с очаговыми разрушениями мембран ядра, митохондрий и гранулярного эндоплазматического ретикулула. Интенсивность катаболических процессов обусловлена наличием в раневом канале токсических компонентов, разрушенных миосимпластов и ионов тяжелых металлов поражающего элемента. На шестидесятые сутки после огнестрельного ранения сохранялась повышенная активность макрофагоцитов, вызванная вяло текущими процессами утилизации разрушенных ультраструктурных компонентов миосимпластов и ионов тяжёлых металлов из поражающего элемента.

რეზიუმე

ჭრილობის არხის მაკროფაგოციტების ულტრასტრუქტურის ცვლილებების დინამიკა ცეცხლნასროლი დაზიანების შემდეგ

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კვლევის მიზანს წარმოადგენდა ჩონჩხის კუნთების სუბმიკროსკოპული არქიტექტონიკის გადაწყობის თავისებურების, ორგანოებისა და უჯრედშიდა მემბრანების ტრანსფორმაციის დინამიკის განსაზღვრა ცეცხლნასროლი ნამსხვრევი ჭრილობის მოდელირების სხვადასხვა ვადაზე.

დაკვირვების ქვეშ იყო 26 ბოცვერი, რომელთაც ჭრილობა მიაყენეს პისტოლეტიდან “ფორტი-12”. ბოცვერები ექსპერიმენტიდან გამოჰყავდათ დაზიანე-

ბიდან 30-ე და მე-60 დღეს. ულტრასტრუქტურული ანათლები, ტყვიის ციტრატით კონტრასტირების შემდგომ, შეისწავლებოდა ელექტრონული მიკროსკოპით ЭМ-125 (სუმი, უკრაინა) ამჟამარებული ძაბვით 75 კვ. გადიდებით 42000-72000.

ჭრილობის არხის ნაწიბუროვანი ქსოვილიდან აღებული მაკროფაგოციტების ორგანოების ელექტრონულ-მიკროსკოპიული კვლევით ჭრილობის მიყენებიდან 30-ე დღეს გამოვლინდა მაღალი ფუნქციური აქტივობის მდგომარეობაში მყოფი მაკროფაგოციტების უხვი რაოდენობა, ორგანოების დისტროფიული და დესტრუქციული ცვლილებები ბირთვის მემბრანების, მიტოქონდრიების და გრანულური ენდოპლაზმური რეტიკულუმის კეროვანი დაზიანებებით. კატაბოლური პროცესების მიმდინარეობა გამოწვეული იყო ტოქსიკური კომპონენტების, დაშლილი მოსიმპლასტების და დამაზიანებელი ელემენტის მიმე მეტალების იონების არსებობით ჭრილობის არხში.

დაზიანებიდან მე-60 დღეს შენარჩუნებული იყო მაკროფაგოციტების მომატებული აქტივობა, გამოწვეული მოსიმპლასტების დაზიანებული ულტრასტრუქტურული კომპონენტების და დამაზიანებელი ელემენტის მიმე მეტალების იონების უტილიზაციის დუნედ მიმდინარე პროცესებით.

MANAGEMENT OF AMNESTIC AND BEHAVIORAL DISORDERS AFTER KETAMINE ANESTHESIA

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General anesthesia may cause damage of the central nervous system and cognitive dysfunction in the postoperative period. The frequency of postoperative cognitive dysfunction – 36,8% [1,2]. The early postoperative cognitive dysfunction is the main predictor of the stable amnesic disorder. It makes worse the patient's quality of life [3-5]. Neuroprotective drugs have an important role in preventing the neuron damage and cognitive dysfunction at the early postoperative period when these changes are potentially reversible [1,2,6-7]. The neuroprotective drugs have a direct influence on memory and mental activity and also increase steadiness of brain to the aggressive effect of hypoxia, injuries, intoxication [2]. A new intranasal form of Noopept (N-Phenylacetyl-L-prolylglycine ethyl ester) was developed by our team at the Department of the medical technology (Zaporizhzhia State Medical University, Ukraine). This work aimed to estimate the neuroprotective action of Noopept and to prove using in the clinic for correction of amnesic and behavioral disorders after ketamine anesthesia.

Material and methods. All experimental studies were conducted in accordance with the “Methodological recommendations for conducting preclinical studies of potential drugs of the State Expert Center of the Ministry of Health of Ukraine”. The studies were performed on a sufficient number of experimental animals. All manip-

ulations were carried out according to the regulation of using of the animals in biomedical experiments (Strasbourg 1986, as amended in 1998). The protocols of experimental studies were approved by the decision of the Ethical Committee of the Zaporizhzhia State Medical University (protocol No. 77 of April 5th, 2018).

50 mature male Wistar-Kyoto rats with body weight of 200-220g were treated in a common laboratory environment (12-hour light cycle, T=+22°C) with free access to water and food. All rats were 6 months old. Animals were obtained from the Institute of Pharmacology and Toxicology of the Academy of Medical Sciences of Ukraine. The duration of the quarantine (acclimatization period) for all animals was 14 days. During the quarantine, every animal was inspected daily (behavior and general condition). Before the beginning of the study, animals that met the criteria for inclusion in the experiment were divided into groups by using the randomization method. Animals that did not meet the criteria were excluded from the study during the quarantine. Cells with animals were placed in the separate rooms.

Ketamine anesthesia was made by the intraperitoneal administration of 100mg/kg ketamine. After recovery from anesthesia the single administration of the drugs in following doses was made: 10mg/kg Noopept by the intranasal way, 0,2mg/kg

cerebrocurin intraperitoneally, 250mg/kg piracetam intraperitoneally. The intact group (the animals without administration of ketamine) received 1ml/100g normal saline intraperitoneally once, and the control group (the animals after ketamine anesthesia) received 1ml/100g normal saline intraperitoneally once. The next day after anesthesia the estimation of motor and search activity by the open field test and in the labyrinth was made during the next 10 days.

Cerebrocurin (Scientific Production Enterprise, Ukraine) is a white, slightly yellowish transparent liquid with pH 6,1-6,4. It contains neuropeptides, including proteins S-100, reelin, nerve growth factor, amino acids [12,13].

Piracetam was used in the form of 20% solution (Arterium, Ukraine).

Noopept (OTCpharm, Russia) is a homogeneous transparent liquid without odor with the low viscosity in the nasal form (Patent # 126979, Ukraine).

Determination of motor activity

Determination of motor activity was carried out by the open field test using arena 80x80x35cm. The animal was placed in the center of the area, and then it has been allowed to walk across the arena freely for 8 min. We estimated general walked distance, general motor activity, structure of activity (high, low activity, torpidity), quantity of freezing and entering in center, distance walked near wall, vertical search activity (quantity of

rearing near wall and free standing), quantity of short and long grooming events, quantity of defecation and urination.

Estimation of reference and working memory

Investigations of memory were carried out with radial labyrinth LE760 (AgnTho's, Sweden). Radial arm maze consists of an octagonal platform (lateral length 22cm) with outgoing 8 radial ray-paths 70cm length and 10cm wide. Each path is closed by the guillotine mechanism independently of one another. All installation was placed at the height of 70cm from the floor. The investigation was carried out in silence.

Starting from the first day animals were placed in the central part with 4 closed rays and 4 opened rays. 200mg of food was put into troughs. Combination of opened and closed rays was individual and regular for each animal. Each animal was trained in food search using visual landmark for 10 min. The experiment was repeated every day twice for each animal. The animals have gotten daily food ration after the experiments. On the 10th day, the animal was placed in a radial labyrinth with 8 opened ray-paths, and in 4 of ray-paths the food was put according to habitual scheme for the individual animal. We estimated the number of referent memory mistakes (first visit the earlier closed ray where animal never found the food), and the number of working memory mistakes (repeated visit the ray where animal found or not found food earlier). Besides, we estimated traversed distance and general motor activity.

Table. Influence of noopept, cerebrocurin and piracetam on rat's behavioral activity and memory after ketamine anesthesia

Index	Intact group	Control group	Ketamine anesthesia+ Cerebrocurin	Ketamine anesthesia+ Piracetam	Ketamine anesthesia+ Noopept
Radial labyrinth					
Number of referent memory mistakes	2	3	2	3	1 ^{#,†}
Number of working memory mistakes	4±1	13±1*	5±1 ^{#,†}	12±1*	2 ^{#,‡,†}
General motor activity, cm ² /s	24380,98±124,4	26867,58±154,5	44862,35±168,5 ^{*,#,†}	27552,12±123,1	34863,66±108,5 ^{*,#,‡,†}
Open field					
Traveled distance, cm	4161,82±29,78	4202,03±77,1	3094,16±34,5 ^{*,#,†}	4013,25±42,36	3916,56±24,48
Number of entering to centre, unit	1	2	1±1	2	1
High activity, %	7,83±1,44	14,83±2,07*	21,83±1,58 ^{*,#,†}	12,22±1,21*	10,50±1,45 ^{#,‡}
Low activity, %	61,71±7,08	65,83±4,03	65,17±3,69	66,22±7,44	78,30±1,59 ^{*,#,‡,†}
Torpidity, %	30,47±6,59	22,34±4,37	13,00±4,64	21,22±5,11	11,20±3,67 ^{*,#}
Immobility, unit	284±35	429±27*	85±21 ^{*,#,†}	434±33*	138±17 ^{*,#,†}
Freezing, unit	284±35	529±27*	374±32 ^{*,#,†}	539±21*	242±28 ^{#,‡,†}
Free distance, cm	59,37±26,31	529,76±21,98*	323,64±88,71 ^{*,#,†}	612,12±55,43 ^{*,#}	226,10±33,44 ^{*,#,†}
Free distance, %	1,43±0,61	11,30±2,67*	8,92±2,01*	12,74±2,0*	6,06±1,20 ^{*,#,†}
Distance near wall, cm	4102,44±289,55	3672,27±312,74	2770,53±281,43 ^{*,#,†}	3700,82±332,90	3690,16±110,34
Standing near wall, unit	4±1	8±1*	5±1 [#]	7±1*	5±1 [#]
Free standing, unit	2	2±1	1	1±1	1±1
Short grooming, unit	4	2*	3	2±1*	3
Long grooming, unit	1	1	1	1	1
Defecation, unit	3	2	2±1	2	1
Urination, unit	1±1	1	1	1	2

notice: * - p<0.05 vs Intact group; # - p<0.05 vs Control group; ‡ - p<0.05 vs Cerebrocurin group; † - p<0.05 vs Piracetam group

Data obtaining and handling

Investigations were carried out at the department of experimental pathophysiology and functional morphology of the Training medico-laboratory center (Zaporizhzhia State Medical University, Ukraine). Experiments have been performed in a well-illuminated room in silence. During the test, the influence of external and internal visual, olfactory and auditory stimuli was excluded. Evaluation of animal's behavior was carried out by the technician without knowledge about belonging animal to the concrete experimental group. Capture and image recording were made by using the color-video camera SSC-DC378P (Sony, Japan). Analysis of video file was made by using software Smartv 3.0 (Harvard Apparatus, USA). All statistical calculations were done by «STATISTICA® for Windows». A significant difference was considered at $p < 0.05$.

Results and their discussion. Evaluation of the specific indexes of open field test showed the negative influence of ketamine anesthesia on the animal behavior (Table). Administration of ketamine didn't lead to a valid change of traveled distance but increased the free distance. In the control group, the rising number of the freezing and immobility of the animals were observed. All these factors indicate a forming of anxiety and excitability of the animals after ketamine anesthesia. Administration of ketamine didn't change a number of free-standing of the animals but led to increasing of standing near the wall in 2 times. Also, the number of short grooming acts was decreased and a number of long grooming was invariable. This fact also indicates a forming of anxiety and excitability of the animals after ketamine anesthesia. Rising of high activity also may be estimated as decreasing of ability to search because a rat makes a lot of unnecessary movements and needs more time for learning a new environment.

When specific training indexes in radial labyrinth were carried out it was revealed that in 10 days after ketamine anesthesia the cognitive dysfunction of animals was present. General activity of animals in the control group hadn't valid differences with intact group. It was established that on the 10 days after administration of ketamine the number of working memory mistakes was increased in 3 times what indicates a breach of amnesic function after ketamine anesthesia.

Obtained data relate to the conception of the postoperative cognitive dysfunction due to ketamine which can lead to forming the stable cognitive deficit and also psycho-emotional disorders – lethargy, fear, anxiety, disorientation, aggressiveness, irritability [14,15]. Administration of ketamine may provoke deposition of amyloid beta-protein and so can provoke long-term cognitive effects [16]. Other reasons for cognitive dysfunction due to ketamine are considered (e.g. inhibition of glutamate transmission, exhaustion of energetic balance and circulatory ischemia) [17].

Our study showed increased free distance in 10 times compare to the intact group after piracetam administration. Piracetam didn't reduce the number of freezing and torpidity. A number of standing near the wall in the piracetam group stayed high and unchanged compare to control group. A number of short grooming acts was at the level of the control group as well. These facts indicate that piracetam doesn't decrease anxiety, fear, excitability and uncomfortable state of the animals after ketamine anesthesia. Also, piracetam didn't have a positive influence on the indexes of cognitive-amnesic functions. The number of mistakes of working and referent memory was not decreased.

Administration of cerebrocurin after ketamine anesthesia reduced anxiety and excitability. So cerebrocurin reduced free distance, number of freezing and standing near the wall. At the

same time, the cerebrocurin didn't have an influence on the animal's comfortability (grooming) and led to rising of high activity compare to intact and even compare with a control group. Cerebrocurin significantly decreased the number of mistakes of working memory and referent memory on the 10th day after ketamine anesthesia.

Protective effects of cerebrocurin on the brain tissue may include its action on the energetic metabolism and homeostasis of calcium, intracellular protein synthesis [2]. Effects of cerebrocurin can have a connection with increasing of neuron plasticity [9].

Intranasal administration of noopept to the animals after ketamine anesthesia led to reducing of anxiety, fear, excitability and had a good effect on emotional status and behavior of the animals. Noopept reduced free distance, a number of standing near wall and immobility compare to control group. Noopept decreased the high (unproductive) activity and increased the low activity.

Noopept significantly reduced the number of working memory mistakes after ketamine anesthesia, and also it was the best result among other investigated compounds. Intranasal administration of noopept also decreased the number of referent memory mistakes. These facts show the high anti-amnesic effect of noopept after ketamine anesthesia and significant neuroprotective effect.

Noopept has an antioxidant action by reducing oxidative destruction of the protein molecules including memory proteins and also by decreasing accumulation of nitrosative stress markers. So noopept can prevent the neuron destruction [18]. Neuroprotective action of noopept may have a connection with the level of anti-inflammatory interleukins [8-11,18-19].

Conclusions.

1. Ketamine anesthesia leads to increasing of anxiety, excitability and worsening of search activity and leads to amnesic dysfunction.

2. Parenteral administration of piracetam doesn't have a significant influence on the animal's behavior, excitability and doesn't have an effect on the animal's memory after ketamine anesthesia.

3. Parenteral administration of cerebrocurin lowers anxiety and also shows some anti-amnesic effect.

4. Intranasal administration of noopept after ketamine anesthesia significantly decreases anxiety and excitability, raises the animal's activity, shows an intensive anti-amnesic effects and increases animal's training ability. Noopept significantly exceeds piracetam and cerebrocurin according to neuroprotective effects.

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SUMMARY

MANAGEMENT OF AMNESTIC AND BEHAVIORAL DISORDERS AFTER KETAMINE ANESTHESIA

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General anesthesia may cause damage of the central nervous system and cognitive dysfunction in the postoperative period. A new intranasal form of Noopept (N-Phenylacetyl-L-prolylglycine ethyl ester) was developed by our team at the Department of the medical technology (Zaporizhzhia State Medical University, Ukraine).

The objectives of this investigation were the study of neuroprotective action of Noopept and to prove using in the clinic for correction of amnesic and behavioral disorders after ketamine

anesthesia. We discovered that the intranasal administration of noopept after ketamine anesthesia significantly decreases anxiety and excitability, raises the animal's activity, shows an intensive anti-amnesic effects and increases animal's training ability. Noopept significantly exceeds piracetam and cerebrocurin according to neuroprotective effects.

Keywords: ketamine, N-Phenylacetyl-L-prolylglycine ethyl ester, noopept, cerebrocurin, piracetam, neuroprotection.

РЕЗЮМЕ

ФАРМАКОЛОГИЧЕСКАЯ КОРРЕКЦИЯ КОГНИТИВНО-АМНЕСТИЧЕСКИХ И ПОВЕДЕНЧЕСКИХ НАРУШЕНИЙ ПОСЛЕ КЕТАМИНОВОЙ АНЕСТЕЗИИ

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Общая анестезия часто вызывает повреждение центральной нервной системы и когнитивную дисфункцию в послеоперационном периоде. Новая интраназальная форма Ноопепта (этиловый эфир N-фенилацетил-L-пролилглицин) разработана сотрудниками кафедры технологии лекарственных средств Запорожского государственного медицинского университета.

Цель исследования - определение нейропротекторного действия Ноопепта и обоснование его использования в клинике для коррекции амнестических и поведенческих расстройств после кетаминовой наркоза.

Обнаружено, что интраназальное введение Ноопепта после анестезии кетаминном значительно снижает беспокойство и возбудимость, повышает активность животного, проявляет интенсивный антиамнестический эффект и повышает способность животного к тренировкам. Ноопепт значительно превосходит пирacetам и цереброкурин по нейропротекторному эффекту.

რეზიუმე

ამნეზიური და ქცევითი დარღვევების მართვა კეტამინური ანესთეზიის შემდეგ

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ზაპოროჟიეს სახელმწიფო სამედიცინო უნივერსიტეტი, ¹ფარმაკოლოგიის კათედრა; ²სამკურნალო-ტექნოლოგიების კათედრა; ³პედიატრიის კათედრა; ⁴„ყოველად წმინდას“ უნივერსიტეტი, ფარმაკოლოგიის კათედრა, როზო, დომინიკა; ⁵V საბავშვო საავადმყოფო, რეანიმაციის განყოფილება, ზაპოროჟიე, უკრაინა

ზოგადმა ანესთეზიამ შეიძლება გამოიწვიოს ცენტრალური ნერვული სისტემის დაზიანება და აღქმითი დისფუნქცია პოსტოპერაციულ პერიოდში.

ნოოპეპტის (N-ფენილაცეტილ-L-პროლიგლიცინ ეთილის ეთერი) ახალი ინტრანაზალური ფორმა შე-

მუშავდა სამედიცინო ტექნოლოგიების დეპარტამენტის თანამშრომლების მიერ უკრაინის ზაპოროჟიეს სახელმწიფო სამედიცინო უნივერსიტეტში. კვლევის მიზანს წარმოადგენდა ნოოპეპტის ნეიროპროტექტორული მოქმედების შესწავლა და მისი კლინიკური გამოყენების მართებულობის დამტკიცება ამნესტიური (ცნობიერებისა) და ქცევითი დარღვევებისას კეტამინის ანესთეზიის შემდეგ.

აღმოჩნდა, რომ ნოოპეპტის ინტრანაზალური მოხმარება კეტამინური ანესთეზიის შემდეგ მნიშვნელოვნად ამცირებს შფოთვის და აგზნებადობას, აძლიერებს "ცხოველურ" აქტიურობას, გამოხატავს ინტენსიურ ანტიამნეზიურ ეფექტს და ზრდის ვარჯიშის უნარს. ნეიროპროტექტორული ეფექტის მიხედვით ნოოპეპტი მნიშვნელოვნად აღემატება პირაცეტამს და ცერებროკურინს.

EVALUATION OF STRUCTURAL CHANGES IN THE AREA OF EXPERIMENTAL MANDIBULAR DEFECT WHEN APPLYING OSTEOPLASTIC MATERIALS BASED ON VARIOUS COMPONENT PERCENTAGE OF HYDROXYAPATITE AND POLYLACTIDE

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Defects in bone structures of different genesis, location and size are quite common in the practice of dental surgeons and maxillofacial surgeons and orthopedic general practitioners, as well as general orthopedic doctors [5,12]. The only effective way to treat such clinical cases is to use a variety of bone substitutes, which can significantly adjust the processes of reparative regeneration and rehabilitation of patients with this pathology [1,2]. A large amount of scientific studies has been devoted to this issue, but the problem has not been fully resolved yet [3,4,6].

Given the significant growth in various contagious infectious diseases, transmitted between organisms regardless of their species, and certain bioethical issues, plastic materials of synthetic origin have become most prevalent [7]. A number of authors emphasize the use of materials based on analogues of the mineral component of bones, particularly hydroxyapatite. [8, 10]

Although in the scientific literature there are references and research data of such compositions, solving the problem of choice is far from complete, since there is still no universal material that would meet all necessary requirements [9]. In this regard, it is relevant to keep searching for new materials that would optimize the processes of reparative osteogenesis and studying the mechanisms of bone tissue regeneration under their influence [11].

In fact, there are few studies that would investigate and compare regenerative processes with percentage compositional variants, and no practical guidance on how to use them has been provided [13].

The main aim is to find out the mechanisms of reparative regeneration of bone tissue in artificially created transverse mandibular defects in rats when applying osteoplastic materials based on hydroxyapatite and polylactide with different component percentage.

Material and methods. Experimental studies were carried out on 114 mature laboratory white male 180-220 g rats fed on a standard vivarium diet in compliance with all the requirements of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1986).

To perform the tasks, all animals were divided into groups (36 rats each): control group - animals whose bone defect was filled only with a blood clot; 1st experimental group - the entire defect volume was densely filled with a block with the ratio of component parts, hydroxyapatite 80% + polylactide 20%; 2nd experimental group was similar, but with the ratio of 50% + 50% [14]. There was also a separate group of intact animals (6 rats) that did not undergo intervention, but only their blood samples were collected to determine the norm of biochemical parameters to be compared.

An experimental defect was created using a spherical dental 2 mm diameter bur according to the method of Chechin A.D. (1989) [15], using a Surgec XT physiodispenser (NSK, Japan) at a speed of 800 rpm constantly cooling the bur with 0.9% saline solution. Perforation defects were created through external access in the submandibular area to the left. Polylactide (PL) - Poly (L-Lactide) Purasorb PL 32 (Netherlands) and hydroxyapatite (HA) Ca₁₀ x (PO₄)₆ (OH)₂ with a particle size of 0.1mm (sintering temperature = 10500C), synthesized at the department of chemical technology of silicates at Lviv Polytechnic university, were used to create the osteo preservation composition (Fig. 1).



Fig. 1. Photo of the obtained composite material

Animals were removed from the experiment by overdosing a solution of sodium thiopental for 7, 14, 21, 30, 90, 180 days. Euthanasia and material sampling were carried out at approximately the same time of day (from 10.00 to 12.00).

A macroscopic study evaluated the overall appearance of the removed jaws and adjacent tissues. Attention was paid to the presence or absence of the inflammatory reaction, the presence of the implantation material, and the degree of its resorption, depending on the timing of the animals' withdrawal from the experiment. An x-ray study was performed using an Intra digital radiograph (Planmeca, Finland) on extracted and preparation jaws of rats. The study was conducted according to the recommendations of Jacobs R. and van Steenberghe D. (1998).

Morphological and morphometric research methods were carried out after the removed jaws had been decalcified in a 10% nitric acid solution for 7-10 days. The decalcified samples were dehydrated in increasing concentration alcohols and embedded in paraffin. Serial histological sections 7-10 microns thick were made from paraffin blocks on a sledge microtome. Histological sections were stained with hematoxylin and eosin.

Morphometric assessment was carried out with the help of inserts of eyepiece micrometer with measurement grids for cytohistostereometric studies. According to the Delesse principle [16], the cross section area, which contains the component under study, is equal to its share in the volume of the object under study. If a system of points is superimposed on the viewing area, which contains several objects, then the ratio of the number of points that fell onto the objects to the total number of points is equal to the specific area of the objects. The calculations were carried out according to the following formula:

$$S_A = r / P,$$

where S_A is the specific area of the object;
r - the number of points that fall onto the objects;
P - the total number of points.

The object of morphometry was the components of the regenerate, the percentages of which were calculated per unit area of the regenerate in three sections of each block. Such a unit of regenerate area was a large square of the eyepiece stereological grid, which contains 100 nodal points, taken as 100% of the regenerate area. By the number of points of the ocular grid, the proportions of the volume relationships of the implant, connective and bone tissues in the bone regenerate were measured.

Statistical analysis of the results was carried out according to the standard method of descriptive statistics using the software package "StatSoft Statistica 10". For the statistical characteristics of the obtained data of each of the experimental animals, the following indicators were calculated: mean value (M) ± standard error (m). Comparison of mean values in different groups was carried out using non-parametric statistical methods (the Wilcoxon test). The difference was considered significant at $p < 0.05$.

Results and their discussion. Analyzing the obtained results, one could find that the regeneration of bone tissue in significant defects in each experimental group of animals showed its own individual tendency and occurred by replacing the defect with a different regeneration tissue.

To confirm the effectiveness of the bioresorbing composition, a group comparative analysis of preparation jaws fixed in formalin solution was carried out in the final study period (180th day).

In animals of the control group, the defect area was clearly visualized; it was possible to identify the rounded edges of the defect, the content of which was represented by non-mineralized, dense tissue that absorbed when pressed (Fig. 2).

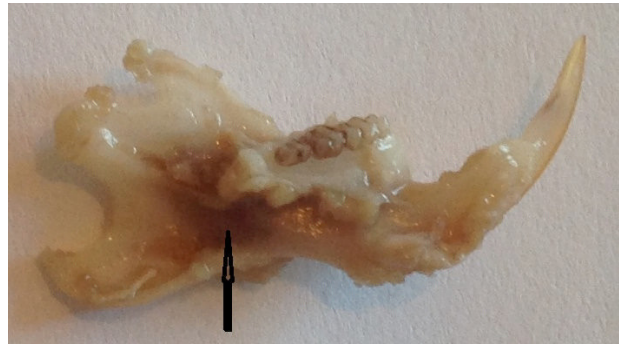


Fig. 2. Photo of specimen of the control group animals on 180 day (the arrow indicates the defect area)

In contrast, in animals of the experimental group 1, it was impossible to identify the edges of the defect, and the entire plane was filled of dense bone tissue, which macroscopically differs from the adjacent one by color and density. Reparative osteogenesis occurred with significant hypertrophy, and the regenerate itself, in the form of a hemisphere, protruded over a healthy intact bone; its surface was smooth. In places, one can find non-resorbed bone implant residue (Fig. 3).

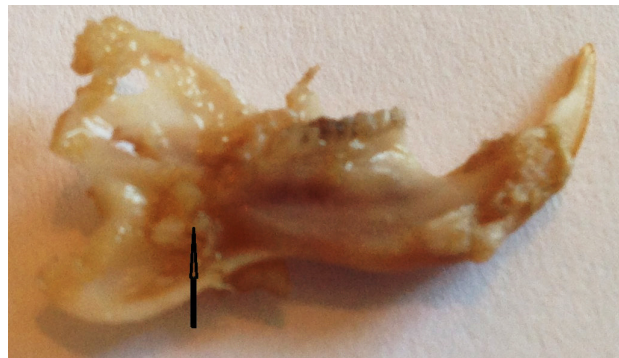


Fig. 3. Photo of specimen of the experimental group 1 animals on 180 day (the arrow indicates the defect area)

When examining the specimen of the experimental group 2 animals on the 180th day, the defect edges were fragmentary, for most of its length its content merged with the intact tissue by color. There were small bumps on its surface. Also, the defect area was detected by the thinning of the bone, especially in the central area. Therefore, one can state that the regeneration processes, unlike those in the experimental group 1, took place with signs of hypotrophy (Fig. 4).

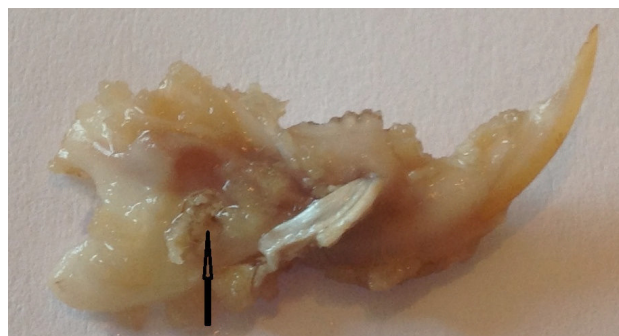


Fig. 4. Photo of specimen of the experimental group 2 animals on 180 day (the arrow indicates the defect area)

To confirm the above mentioned conclusions about the regeneration ways of the mandibular defects that are identical by volume but different in terms of healing conditions, the next task was to establish a correlation with histomorphological changes in the defect area.

Using morphometric methods and calculating the percentage values of the main components of the regenerate (Fig. 5), one found that in the control group on the 7th day of the study, the cavity was almost completely filled with tissue detritus, the amount of which gradually decreases. However, the results of disintegration disrupt the direction of proliferation and spatial orientation of osteogenic cells. This leads not only to a slow-down in osteogenesis, but also to its distortion.

A complete regeneration of the bone defect with restored bone architectonics was not observed even after 180 days of the study. At that time, the regenerate consisted of 66.5% of osteoid tissue and 25.0% of cartilage tissue. Fibrous bone tissue prevailed exclusively along the periphery of the regenerate and made up only 8.2%. A compact plate was noticed to be formed along the edges of the defect, separating the intact bone from the regenerate. Such data led to the conclusion which coincided with the opinion of others scientists [17,18] that the regeneration of large in volume defects of the mandible is characterized by the formation of non-mineralized tissue (osteoid), which is similar in structure to the organic bone matrix.

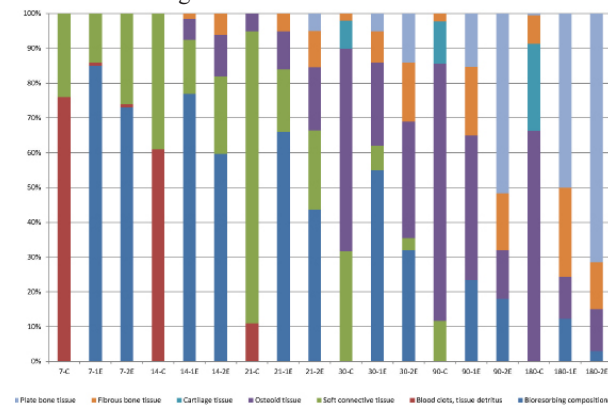


Fig. 5. Morphometric characteristic of reparative bone regeneration of mandibular defects

Based on these results, the need to create more optimal conditions for reparative osteogenesis, which can be achieved by filling the entire defect volume with appropriate substitutes that have a certain biological effect, is obvious.

A simple mechanical filling of the defect cavity with both options of the bioresorbing composition based on hydroxyapatite and polylactide reduces the volume of a blood clot and therefore decreases the scale of destruction of the surrounding tissue defect and has a preventive effect on its suppuration; none of the experimental animals of these groups had inflammatory complications.

An important property of such implants is their degree of biodegradation that is the property of gradual resorption caused by autogenic biologically active compounds and macrophages in the damage area and gradual replacement of the damaged sections with bone tissue. In both experimental groups, osteogenesis occurred in the direction from the periphery to the center due to the proliferation of cancellous bone osteoblasts. As a result of the biooste implant resorption, the loose connective tissue grew in and gradually transformed into the fibrous one, skipping the endochondral osteogenesis phase. The dissolved and released

mineral part of the implant was the basis for mineralization of the formed organic bone matrix.

However, certain differences in the general pattern of reparative osteogenesis described above in animals of experimental groups were observed, depending on the implant composition, which, as confirmed by these studies, had a different degree of biodestruction.

The increased proportion of hydroxyapatite to 80% prolonged the period of its degradation and by 180 day the implant proportion in the regenerate of the 1 experimental group animals had made up 12.7% ($p < 0.05$ to the 2 experimental group). The proportion of mature bone tissue was 51.7% ($p < 0.05$ in the control group), and the presence in the regenerate of 12.5% ($p < 0.05$) of osteoid tissue and 26.5% ($p < 0.05$) of the fibrous tissue indicates the remodeling stage of the primary bony callus.

With the proportion of hydroxyapatite, which dissolves more slowly, decreasing to 50%, as noted above, the degree of bone implant biodegradation increased. Due to such adaptation of the biodegradation degree to the regeneration processes, osteogenesis in the 2 experimental group animals was faster. Morphometric studies showed that on 180 day, mature lamellar bone tissue made up the majority of the regenerate, at 70.0% ($p < 0.05$ to the 1 experimental group). A small proportion of osteoid tissue (11.7% with $p < 0.05$) and fibrous bone tissue (13.3% with $p < 0.05$) indicated the completion of the remodeling process of the primary bone callus.

The X-ray examination confirmed the indicated morphological data and revealed an important difference in the formation of the regenerate. This difference was not established during the histological examination of the microslides taken from mandibular defect tissues of experimental animals.

The data obtained from the mandible X-ray of the control group animals correlated with the data of the histomorphological study; on 180 day the defect area was clearly visualized and was more contrasting along the edges, which indicated the formation of the compact plate, while the veil in the central area had a low uniform contrast, which indicated that the defect was filled with a low mineralized tissue (Fig. 6).



Fig. 6. X-ray of control group animals' mandible, day 180; g - the defect projection

Meanwhile, as regards the experimental group 1 animals X-ray, the defect contour was impossible to identify, but the area of increased X-ray contrast, which dominated over the defect and indicated hyperproduction of highly mineralized tissue, was. It should also be noted that to determine the presence of bone trabeculae and cavities in the regenerate was difficult, which indicated the incomplete formation of mature tissue. In some places one could detect focal growth of even greater X-ray contrast, which indicated the presence of implant residues (Fig. 7).

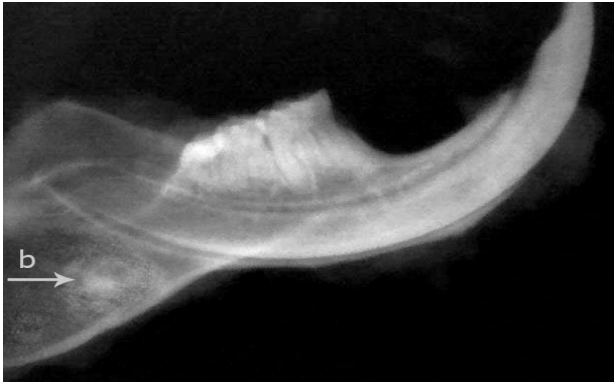


Fig. 7. X-ray of experimental group 1 animals' mandible, day 180; b - implant material in the defect projection

When the defect was filled with the bioresorbing implant with a rapid biodegradation degree (experimental group 2) on 180 day, according to X-ray data, one could ascertain the completion of osteogenesis, since the defect edges were impossible to detect, and bone trabeculae and cavities could be clearly identified. However, the regenerate X-ray contrast was not identical; along the periphery, it practically did not differ from a healthy tissue, but in the central areas it was lower, indicating the regenerate thinning. It was also possible to detect only single point traces of increased contrast, which indicated the implant residue (Fig. 8).



Fig. 8. X-ray of experimental group 2 animals' mandible, day 180; b - implant material in the defect projection

Conclusions. The presented data allow us to conclude that the use of osteoplastic material with a longer period of its biodegradation (with a large percentage of the mineral component) contributes to filling the defect with bone tissue, but slows down the processes of the final formation of a mature secondary bone scar, yet allows even with hypercorrection to restore the anatomical form of the lost area. Therefore, as regards the jawbone, the use of this material is recommended for a defect in the alveolar ridge, mainly in extraction sockets, when one needs to avoid atrophy and to create conditions for prosthetic treatment.

Osteoimplant with a faster biodegradation period (with a lower percentage of mineral component) allows structuring collagen fibers in the regenerate in the early stages of healing, which optimizes the formation of an organic bone matrix and promotes its faster mineralization. However, despite the complete filling

of the defect with the mature bone, signs of incomplete recovery of the anatomical form of the lost bone area and the formation of residual defects were observed. Therefore, such bone substitutes can be better used for insignificant defects or when the restoration of the anatomical form is not essential, mainly for defects of the body and mandibular branches.

Prospects for further research. It may therefore be claimed that using the proposed bone substitutes in clinical practice is promising and important, which will significantly expand the possibilities of modern reconstructive and restorative maxillofacial surgery, and more thoroughly address the issues of patient rehabilitation to the next prosthetic treatment.

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SUMMARY

EVALUATION OF STRUCTURAL CHANGES IN THE AREA OF EXPERIMENTAL MANDIBULAR DEFECT WHEN APPLYING OSTEOPLASTIC MATERIALS BASED ON VARIOUS COMPONENT PERCENTAGE OF HYDROXYAPATITE AND POLYLACTIDE

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The main aim is to find out the mechanisms of reparative regeneration of bone tissue in artificially created transverse mandibular defects in rats when applying osteoplastic materials based on hydroxyapatite and polylactide with different component percentage.

Experimental studies were carried out on 114 mature laboratory white male 180-220 g rats that were divided into groups: control group - animals whose bone defect was filled only with a blood clot; 1st experimental group - the entire defect volume was densely filled with a block with the ratio of component parts, hydroxyapatite 80% + polylactide 20%; 2nd experimental group was similar, but with the ratio of 50% + 50%. Morphological and morphometric research methods were carried out after the removed jaws had been decalcified in a 10% nitric acid solution. An x-ray study was performed using an Intra digital radiograph (Planmeca, Finland). Statistical analysis of the results was carried out according to the standard method of descriptive statistics using the software package "StatSoftStatistica 10".

The use of osteoplastic material with a longer period of its biodegradation contributes to filling the defect with bone tissue, but slows down the processes of the final formation of a mature secondary bone scar, yet allows even with hypercorrection to restore the anatomical form of the lost area. Therefore, the use of this material is recommended for a defect in the alveolar ridge, mainly in extraction sockets, when one needs to avoid atrophy and to create conditions for prosthetic treatment. Osteoimplant

with a faster biodegradation period allows structuring collagen fibers in the regenerate in the early stages of healing, which optimizes the formation of an organic bone matrix and promotes its faster mineralization. Therefore, such bone substitutes can be better used for insignificant defects or when the restoration of the anatomical form is not essential, mainly for defects of the body and mandibular branches.

Keywords: bone regeneration, hydroxyapatite, polylactide.

РЕЗЮМЕ

ОЦЕНКА СТРУКТУРНЫХ ИЗМЕНЕНИЙ В ОБЛАСТИ ЭКСПЕРИМЕНТАЛЬНОГО МАНДИБУЛЯРНОГО ДЕФЕКТА ПРИ ПРИМЕНЕНИИ ОСТЕОПЛАСТИЧЕСКИХ МАТЕРИАЛОВ НА ОСНОВЕ ГИДРОКСИАПАТИТА И ПОЛИЛАКТИДА С РАЗЛИЧНЫМ КОМПОНЕНТНЫМ ПРОЦЕНТОМ

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Целью исследования явилось определить механизмы репаративной регенерации костной ткани в искусственно созданных сквозных дефектах нижней челюсти с применением костно-пластического материала на основе гидроксиапатита и полилактида с различным процентным содержанием компонентов.

Экспериментальные исследования проводили на 114 половозрелых лабораторных белых крысах-самцах массой 180-220 г, которые были разделены на группы: контрольная - животные, костный дефект которых заполняли кровяным сгустком; первая исследовательская группа - весь объем дефекта плотно наполняли блоком гидроксиапатита и полилактида в соотношении 80% к 20%, соответственно; вторая исследовательская группа - аналогично, однако в равном соотношении. Морфологические и морфометрические методы исследования проводились после декальцирования удаленных челюстей в 10% растворе азотной кислоты. Рентгеновское исследование выполнено посредством цифрового рентгенографа Intra (Planmeca, Финляндия). Статистический анализ результатов проводили согласно стандартной методике описательной статистики с использованием программного пакета «Stat Soft Statistica 10».

Использование остеопластического материала с более длительным периодом его биодegradации способствует заполнению дефекта костной тканью, однако замедляет процессы окончательного образования зрелого вторичного костного рубца и позволяет даже при гиперкоррекции восстановить анатомическую форму утраченной площади. Исходя из вышеизложенного, использование этого материала рекомендуется при дефекте альвеолярного отростка, в основном, в экстракционных патрубках, когда необходимо избежать атрофии и создать условия для протезирования. Osteoimplant с более быстрым периодом биодegradации позволяет структурировать волокна коллагена в регенерате

на ранних стадиях заживления, что оптимизирует образование органического костного матрикса и способствует его более быстрой минерализации. Следовательно, такие заменители кости могут быть лучше использованы для незначительных дефектов, когда восстановление анатомической формы не является существенным, в основном, для дефектов тела и ветвей нижней челюсти.

რეზიუმე

სტრუქტურული ცვლილებების შეფასება ექსპერიმენტული მანდიბულარული დეფექტის არეში ჰიდროქსიაპატიტის და პოლილაქტიდის სხვადასხვა კომპონენტური პროცენტით ოსტეო-პლასტიკური მასალების გამოყენების პირობებში

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კვლევის მიზანს წარმოადგენდა ძვლის ქსოვილის რეპარაციის მექანიზმების განსაზღვრა ქვედა ყბის ხელოვნურად შექმნილ გამჭოლ დეფექტებში ძვალ-პლასტიკური მასალის გამოყენებისას ჰიდროქსიაპატიტის და პოლილაქტიდის საფუძველზე კომპონენტების სხვადასხვა პროცენტული შემცველობით.

ექსპერიმენტული კვლევა ჩატარდა 114 ზრდასრულ, 180-220 გრ მასის თეთრ მამრ ვირთაგვაზე, რომელთაც გაყოფილი იყო ორ ჯგუფად: საკონტროლო –

ცხოველები, რომელთა ძვლის დეფექტი შეივსო სისხლის კოლტით; პირველი საკვლევი ჯგუფი – დეფექტის მთელი მოცულობა მჭიდროდ ივსებოდა ჰიდროქსიაპატიტის და პოლილაქტიდის ბლოკით თანაფარდობით, შესაბამისად, 80% და 20%; მეორე საკვლევი ჯგუფი – ანალოგიურად, მაგრამ თანაბარი რაოდენობით. მორფოლოგიური და მორფომეტრული კვლევა ჩატარდა ამოღებული ყბების აზოტმუავას 10% ხსნარით დეკალცინირების შემდეგ, რენტგენოლოგიური კვლევა - Intra ციფრული რენტგენოგრაფიის (Planmeca, ფინეთი) მეშვეობით. შედეგების სტატისტიკური ანალიზი განხორციელდა აღწერილობითი სტატისტიკის სტანდარტული მეთოდით პროგრამული პაკეტით «Stat Soft Statistica 10».

ოსტეოპლასტიკური მასალის გამოყენება მისი ბიოდეგრადაციის უფრო ხანგრძლივი პერიოდით ხელს უწყობს დეფექტის შევსებას ძვლოვანი ქსოვილით, მაგრამ აწვდის ზრდასრული მეორადი ძვლოვანი ნაწიბურის საბოლოო წარმოქმნის პროცესს, თუმცა, ჰიპოკორექციის დროსაც კი იძლევა დაკარგული ფართობის ანატომიური ფორმის აღდგენის შესაძლებლობას. ზემოაღნიშნულიდან გამომდინარე, ამ მასალის გამოყენება რეკომენდებულია ალვეოლური მორჩის დეფექტის დროს, როდესაც აუცილებელია ატროფიის თავიდან აცილება და პირობების შექმნა პროთეზირებისათვის. ოსტეოიმპლანტი ბიოდეგრადაციის უფრო სწრაფი პერიოდით იძლევა რეგენერატში კოლაგენის ბოჭკოების სტრუქტურირების საშუალებას შესორცების უფრო ადრეულ სტადიებზე, რაც ხელს უწყობს ორგანული ძვლოვანი მატრიქსის წარმოქმნის ოპტიმიზებას და მის უფრო სწრაფ მინერალიზაციას. აქედან გამომდინარე, ძვლის ასეთი შემცველები შეიძლება უმნიშვნელო დეფექტების დროს უკეთ იყოს გამოყენებული, როდესაც ანატომიური ფორმის აღდგენა არ არის არსებითი, ძირითადად, სხეულის და ქვედა ყბის ტოტების დეფექტების დროს.

STUDENTS POPULATION'S ATTITUDE CONCERNING ENVIRONMENTAL ISSUES IN GEORGIA

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Environmental pollution is one of the most important global threats in the world. The immediate objects of pollution are land, water, soil, air [1,2]. The risk of mortality is mostly caused from environmental risk factors: contaminated water, polluted air, industrial and household waste. Human health can be significantly improved by reducing the impact of factors such as pollution, hazardous work environment, ultraviolet radiation, noise, threats to agriculture jobs, climate and ecosystem [3,4].

Environmental pollution sources can be divided into three groups: natural, industrial and household. Natural sources of pollution are endogenous (earthquakes, volcanism and so on) and exogenous factors (climate, wind, ice, soil erosion, flooding of rivers etc.) and anthropogenic sources belong to industrial and domestic pollution factors.

Positive attitude towards the environmental pollution are important for sustainable development of the country [5]. This

is particularly important for Georgia, the geopolitical location of which further aggravates the situation [6,7]. In Georgia this is important in terms of air and soil, because very little part of the enterprises operate in our country, the main source of pollution is the exhaust of the car and the situation is worsening by mangled trees. As for the water resources, the conservation of household and industrial wastes in the river leads to pollution of water resources and also soil contamination, as water is often used for soil irrigation, which is obviously an unhealthy fashion on the ground water that is finally reflected on human health. The most important of these three factors is soil contamination, as it is possible to mitigate air pollution by planting trees and filtering water resources, but the soil is a practically undetectable component of the biosphere on which is depended the condition of human life. In this regard, our land is noteworthy, as well as the problem of clogging, absence of trails and uncontrolled use of poisonous chemicals [8].

Many studies have focused on students' attitude, interest and behavior towards environmental problems [9-11]. Studies have confirmed that students have positive behavior and attitude towards the environment [12]. Moreover, they express high emotional attitudes towards the environment, have high interest in environmental problems and are willing to find ways of their solution [13,14]. Some research show that students have a positive environmental impact, but their knowledge is inadequate [15].

The purpose of the study was to determine the student's attitude towards environmental problems and to analyze the behavioral characteristics of the students about the solutions. The main tasks of the research are:

- Study student's involvement in environmental activities;
- Overview of environmental legislation of Georgia;
- Search for ways to eliminate problems related to environmental protection by students.

Material and methods. Two focus groups and 4 in-depth interviews were conducted within the qualitative research. In order to study the issue deeply, we used a triangular approach. We talked to the expert at the beginning of the research. The target group is students who are studying at the university level of bachelor's and master's level. Students were selected through snowball sampling method.

Primary data was used, where we have collected data our self, using the interviews. Units of data analyses were words and phrases, where we recorded the interview, and created a transcript afterwards. Interviews have been conducted in May-June, 2018.

The study was approved by the Ethics Committee of the Ilia State University. The protocol was in accord with the declaration of Helsinki. We conducted interviews and tried to protect the ethical standards. To avoid any difficulties, we had explained them the aim and the objectives of the research and after that we took the permission to record an interview on the audio tape. We have taken informed consent from each participant. All respondents had the opportunity to stop interviewing at any time. The results of this research are confidential and analyzed only in general form. All these actions aim to ensure the confidentiality of the respondents. The researchers were obliged to protect all the rights and ethical standards.

Results and their discussion. Expert Survey Results

We have received interesting information on ecological problems in Georgia based on the survey of experts. According to experts, there is a difficult ecological situation in Georgia. In this respect, each region is characterized by different problems.

"Environmental approaches should be divided according to regions, as only one approach can not prevent the problem in

both, mountain and bar. If there is a problem of emissions in the city, we can not say the same thing about the village where the air is made through natural corridors.

Waste management in major cities was named as the main ecological problem. The situation is complicated by the fact that most of the existing enterprises in Georgia were built prior to 30-40 years, which are not equipped with filling equipment. Wrong construction engineering activities and unforeseen considerations of the competent persons are a great threat to the public.

Tbilisi is located in a high-seismic environment. Some parts of buildings are not adapted to the seismic conditions. Earthquake can cause catastrophic consequences. "

Experts especially stressed the problem of water pollution.

"We are eating the food which is coming from soil and the soil itself is getting irrigated through the rivers, so there is a high chance that the food we're getting from the soil is dangerous for our body.. Many of the rivers are polluted so that the fish caught in it may have a negative effect on people. For example, underground rivers in Chiatura, which constitute a large reservoir of water, are dirty by the heavy metals of industrial enterprises which can be dangerous for human being. In the Marneuli region a large area is irrigated by the river Kazretula. Water coming from Kazreti industrial enterprises will flow into the river. Nobody knows about the content of heavy metals in the products obtained from Marneuli. "

According to experts, only appropriate infrastructure for the prevention of environmental problems is not a decisive factor. Also awareness of the population is important.

"In some countries, there is no proper infrastructure at certain villages, but the population is burning a trash and It physically no longer remains in the environment. However, there are territories where infrastructure exists, but the population is rubbing in the river or in the caves and trying to get rid of the trash more easily. As a result, drinking water is getting polluted.. That is why it is necessary to provide information to the population about the damages inflicted on its vital environment as a result of such actions".

According to the experts, social media, mass media has the biggest role in raising the level of awareness of the population.

"Social networks, advertisements, TV programs are more likely to change anything than booklets and field specialists, because almost no one can read the booklet, in the meetings are coming only those, who are interested".

Family and school has a big role in raising the level of human consciousness.

"The child is initially formed into a family as a person and then at school where the ecological education should be taught. The subject of ecology consists of the knowledge that the person has to study from the earliest age and behave accordingly. The Ministry of Education should spend more on ecological education. Students and the scientific community's involvement is important for prevention of environmental problems, since students blithely performing useful work and are not biased, and the the members of scientific community are the competent persons and they can provide information to the public, in order to increase the level of awareness, as well as government – for the right decisions."

Focus group results

Within the framework of the survey, two focus groups were held. Those focus groups included Bachelor's and Master's students from Ivane Javakhishvili Tbilisi State University. On the question - what does environmental protection mean, we have received more or less different and interesting answers from

the respondents. For some of them, environmental protection is part of a civil obligation, for others environmental care is maximal protection and consideration of regulations, for some it's a warning of natural resources, care for ourselves and for some of the respondents desire of protecting the environment is coming from internal culture:

(Lela) - "In my opinion, environmental protection is a civil obligation"

(Johnny) - "Environmental protection means maximizing protection and consideration of laws, and regulations developed by the state and superior institutions."

(Maka) - "I agree with the opinion of the previous two respondents, and I want to add that environmental protection is, first of all, a warning to the natural resources."

(Vakho) - "In my opinion, environmental protection is primarily not environmental, but also the care shown towards us, because when we are disturbed by environment, we are facing the death penalty for the first time."

(Leila) - "In my opinion, environmental protection implies the inner culture, what kind of person you are and what kind of person you are growing up in the environment, I mean, you do not have to contaminate the environment, because it is reflected negatively."

On the question - how important this topic is, answers are almost the same. Each participant of the focus group emphasized the importance of this topic, as these are the problems that people face each day and affect their vital environment:

(Nina) - "Of course this is the topic of concern, first of all because environmental cleanliness and ecological cleanness deals with our health and our lives."

(Tornike) - "Today it is a big problem in Georgia, the main streets of Tbilisi are not so polluted as external areas that seriously harm people's health."

What are the problems related to environmental protection in Georgia? Which one do you consider more important? Why? The answers were very interesting because the respondents demonstrated different opinions. The following problems were identified: sea pollution, emissions, uninterrupted landfills, timber picking, water and air pollution, household waste and construction of power plants:

(Maka) - "I'm talking about a personal example, I'm from Batumi and sea pollution is one of the most problematic problems for me in Georgia, you know, oil is imported in Batumi port, and often the oil is swept away and the remains remain in the sea, and it's all about the unbearable smell."

(Tamuna) - "Due to the fact that here are so many cars which cause the danger to human health, I want to point out vehicle emission"

(Johnny) - "In my opinion, it is quite a real problem for timber pickups, especially in late autumn when the winter comes, illegal cutting of timber is starting and this creates health problems for us, because oxygen is not provided with sufficient dose."

(Faye) - "For me, water and air pollution is the biggest problem, because it is two of the factors we have every day to touch, and I think our health is mostly affected by polluted water and air."

(Salome) - "In my opinion, the biggest problem is household waste, especially I'm talking about polyethylene parks, because their disappearance from nature may take centuries and it is very dangerous for both the environment and for humans ..."

(Vakho) - "The main problem is the construction of HPPs, as it seems to be a source of clean energy and not damage the environment at all, but if we look closely, construction of new HPPs causes huge problems, some think that the cost of the economy

is to destroy the environment, The main thing is the economic and not the ecological factor, because they believe that ecology is a future problem and it can not cause any problem now. "

Respondents think that the environment in Georgia is unfavorable. There are different rural and urban problems. For some respondents, problems in the city seemed to be more important, and for others on the contrary. Air pollution in the city is actual, and in the village - due to lack of infrastructure, unpacked rubbish groves. Both equally threaten the vital environment of the human being.

(Lela) - "... I do not agree that we're mostly facing these problems in towns, I think the situation is worst in the villages where there are no rubbish bins and the locals are lying in the river or in the ravine."

(Faye) - "In my opinion, these problems are more acute in the city and the most important of them is exhaustion, caused by the abundance of transport."

(Johnny) - "In my opinion, the polemics about the difference of the city and the village are illogical, and we are dealing with different problems, both in ecological and rural areas, and in fact there are other problems in the village and in the city, and no one can overturn the other."

As for environmental measures, most of the respondents had information about them, but most of them did not take part in any environmental action that some of them have accused of lack of time and partially ill-informed. Some of the respondents have stated that they have participated in the so-called "Saturday" and one-time participation in activities such as mass cleaning activities:

(Tamuna) - "It would be nice to have environmental activities in Georgia, but I did not come to know about their existence. I would work with pleasure, even in the morning, because no students abroad are unemployed, students are standing in the morning and taking care of their city, that is acceptable for me, but unfortunately in Georgia I haven't heard about such kind of activities yet."

(Johnny) "Unfortunately, I have never taken part in environmental events because of lack of time, but I have heard about such activities as" No Hudson "," Panorama Tbilisi "," Protecting Hippodrome "," which is very relevant today. In fact, the most active organizations are: " Green Fist "and" Partisan Gardeners".

(Vakho) - "I've heard and I have participated in many activities, for example," No Hudonhesi. "Finally all of our initiative ended with failure, but the good thing was that the level of awareness was increased in society."

(Leila) - "We have put some trees in school on Saturday, perhaps because they have grown up more and more of the trees and healthy environment for the future generation, as for the events ... (silence) I did not participate in ..."

As for NGO'S, the majority of students did not have information about them, they are mostly familiar with the organizations that are formulated by students. Some have expressed mistrust towards NGOs because they do not actually do the job:

(Giorgi) - "I have heard about some of the organizations on TV that clean up some areas, but in my opinion it is their PR, some small place will be cleaned up and then it's going to be a long report on television."

(Nina) - "Green Fist", is one of the most active organizations in Georgia. It's created by students and one of the leaders is from our faculty who is very concerned about our ecological condition. They were one of the first ones who opposed the construction of the hydro power plant and other hydroelectric power plants. "

Respondents think that the policy pursued by the state is not

effective in this regard. In part, the sanctions that are established are not functioning because there is no monitoring service. Part of the opinion is that public awareness level is low and it creates a problem. In some cases, the state emphasizes the social-political issues and the environmental issues remain in the background.

(Salome) - "As for the policy pursued by the state, we have a relevant law that punishes environmental pollution, but in reality I have not seen such an action, it is possible that once in a while people have been fined, but I think that the law must be carried out in life itself."

(Otar) - "In my opinion, there is an attempt by the state, but it will not be effective until the public consciousness does not rise."

Most of the focus group participants underscored the level of consciousness of people that are important for individual actions. In order to specify the issue, we asked them whether they needed to raise awareness of human beings and how can a citizen's awareness be increased in environmental protection?

(Nina) - "In my opinion, it is impossible to eliminate this problem without raising awareness of the human person, each person should consider that it is a problem that will reflect on his health, but what can you talk to a person, who kicks out of his own window. Human consciousness has to be raised through media, school, university, especially the childhood should be the creation of human consciousness in this regard, the role of the family is also important."

Despite of the fact that the majority of respondents have a great understanding of the level of consciousness, the importance of the law as an alternative means of eliminating environmental problems has been emphasized. That's why we were interested in the information about Georgian legislation and found that many of them knew about this legislation. However, their care is not explained by law, but from their mentality and culture. Respondents think that establishment of the law is nothing than control and the appropriate sanctions.

(Otar) - "In my opinion, no results have been taken because throwing out the cigarette in the streets is control by the police, but In fact It's a stupid lever, because the passenger policeman is unable to notice a person throwing a cigarette in the street. Some effective changes should be made, we can take an example from other countries, where these issues have been disciplined."

Finally, we asked the participants of the focus group how the ecological condition of Georgia is concerned - most of them believe that environmental problems in Georgia are in such a way that it is impossible to improve the situation, but it is possible to control the situation. Also, from their point of view, it is expected to change the outlook of the society, that is, to realize that by damaging the environment, they have a negative impact on their own health.

(Johnny) - "I hope that the ecological problems in our country and the world around will be improved, I think that students and younger generation have a bigger role, and the change of generations depends on the change of mentality so that in older generations it is more difficult to change the mentality, change the consciousness ... That is why I think the main strength is in youth".

In-depth interview results

Students, interviewed by depth interview method, think, that environmental protection means protection of purity and infrastructure, care for natural resources and planting trees. All this should be determined by the inner culture of the human being, as they are putting environmental protection and inner culture on the same level.

According to the respondents, The following problems are relevant in Georgia: forest cover, air and water pollution, household waste.

According to the respondents, the main problem for the population of the cities in Georgia is vehicle exhaust, and in the villages - the failure of the infrastructure, in particular, there are no trash bins and the locals are throwing trash on a messy landfills. Such landfills are mainly located at the beginning and end of the village, in the ravines, or in the rivers. The population is not informed about what kind of damage it is causing to its vital environment.

(Rati) - "...There are different problems in villages and districts. If the exhaust is disturbed in the city, in the villages and regions most of the wastes are problematic. Nature and water are contaminated. In a lot of villages people drink water from the source and prove that they can not drink such a clean water in other place, but they do not think about the trash and disaster inside and while cleaning, someone will definitely drink and that will harm the human organism."

(Tako) - "Of course. There have been many cases when people got poisoned and there were reports on television, that people are poisoned by water and it brings disastrous results. In addition, the crop produced on polluted soil will have a bad effect on the human health, statistically I do not what is the situation we are facing, but it is a fact, that we are threatening our lives with our unadvised actions. On "Facebook" I saw that global warming has already begun and the temperature rises by 2 degrees."

According to the respondents, solving environmental problems by state is more or less useful, some type of activities has been observed, but very rarely, so much more needs to be done. Despite the publish of the "Waste Management Law" in January 2015, we cannot say that this law has greatly influenced the current situation, because the enforcement and monitoring of the law are underway.

(Oto) - "There is a law about environmental protection but it is unreasonable. In recent years, fines have been imposed on pollution, but in my opinion it is not effective, because the country is more pressing for a number of social and political problems and the government does not have time for environmental problems, however, It really has to be taken care of. I think the state must strictly control the law, because we are a nation, which needs to be controlled, because without it we will not be able to take measures accordingly, but if each of us does not understand our duty towards nature, the state alone can not be helpful."

Part of the respondents positively assessed the work of NGOs with this profile, while partially demonstrated the distrust and negative attitude towards them, which was explained by stereotypes about NGOs, that they are not capable of doing their job properly.

(Faig) - "I know that there are couple of organizations, such as "green's". This organization calls upon citizens not to pollute the environment, to take care of it and two times a year be actively involved in cleaning, plant trees and greenery the environment. Unfortunately, other organization's names are not known for me, but I often see such kind of non-governmental organizations by television or Internet.

(Oto) - "I do not know any non-governmental organization that is taking care of environmental protection, so I think that they do not have any activity on their side, so I cannot say anything positive about these NGOs, more of the empty words are coming from most of them. but I have some information about student movements. For example, student movements of the State University such as "green fist" and "partisan gardeners" have periodic demonstrations regarding environmental protection"

As for the mass media, each respondent emphasized its importance in terms of influencing society. However, it was also noted during the conversation, that it does not use its capabilities.

(Tamar) - "The role of mass media, as the fourth government, is the biggest to raise awareness of the people consciousness, but unfortunately in television TV series have a much higher rate than the various cognitive programs. It is necessary to correct all this, because TV channels such as" BBC" are actively working in this direction. In general, mass media has a huge impact on society and the workers in this field should realize their responsibility.

Respondents think, that education is very important in terms of raising awareness of human consciousness. In their opinion, people should learn the importance of environmental protection from their parents. It is also important to get both formal and informal education, which implies the study of environmental issues in schools and universities. According to the respondent's reply, it would be nice if one special lecture about environmental protection will be held at universities.

(Oto) - "In my opinion, it's essential for everybody to learn in childhood about how to treat the nature. Couple of years ago, as far as I know, ecology was one of the subjects in a school. I think it's a necessary subject. There should also be some activities, public lectures where environmental experts will talk about why the environment should be protected. It is also important to get educated in family, get advice from parents, friends and relatives about environmental protection.

Respondents have also mentioned fabric parks, cotton packets as very useful things for the environment. It is a very pleasant fact that each and everyone of them have information about it and support policies that require replacement of polyethylene parks especially with the fabric parks.

(Faig) - "Fabric parks and Cotton Packs are quite effective in terms of environmental protection, as they replace the polyethylene parks. It is well known fact that the destruction of the polyethylene park needs hundreds of thousands of years, accordingly, fabric parks are necessary. Unfortunately, it is not widely used in Georgia. Most stores in the country use polyethylene park, probably it will be quite difficult to replace them with a fabric park."

Respondents also talked about renewable energy sources and noted their advantages as well, but it costs a lot.

(Tamar) - "Yes I have heard that it is very good, in Georgia probably solar energy will be used. I see the so-called solar batteries that are very economical, but the use of these resources is still linked to money and probably Georgia is not yet ready for that."

(Faig) - "Yes I have heard that they are actively and effectively in developed countries. In developing countries, for example, in Georgia, there are no funds available, even though water is quite large and cannot be used in this regard."

Conclusion. According to the respondents, the most important problem in Georgia is air, water and soil pollution. The contaminated environment is directly related to worsening of human health. The participants have favorable attitudes toward the environment, but they are less involved in environmental activities. Basically these measures are limited to the so-called one-time campaigns. The part of the respondents who have never participated in such a measure justifies their actions by the fact that the environment is not delayed by its individual actions. According to the respondents, the state activities in this profile is less helpful. Respondents think that actions taken by govern-

ment for solving problems of environment are less useful, also the monitoring of the law is less and it is necessary to tighten it.

Education is very important in terms of raising awareness of human consciousness. It emphasizes both informal and formal education, which envisages studying environmental issues in schools and universities.

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SUMMARY

STUDENTS POPULATION'S ATTITUDE CONCERNING ENVIRONMENTAL ISSUES IN GEORGIA

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The aim of this study were to investigate the attitude of university students on the environmental issues, analyze the information received and develop measures for the participation of students in environmental health-improving and preventive activities. The qualitative study was conducted by using face to face interviewing method with university students. Environmental pollution is one of the most important threats in Georgia and around the world. The immediate objects of pollution are land, water, soil, air, which directly affects human health and worsens it. The participants have favorable attitudes toward the environment, but they are less involved in environmental activities. Basically these measures are limited to the so-called one-time campaigns. Based on the results, it is recommended the development of integrated measures and schemes to stimulate student participation in the implementation of socially active algorithms to improve the environment.

Keywords: environmental pollution, healthy lifestyle, environmental attitudes, environmentally responsible behavior.

РЕЗЮМЕ

ОТНОШЕНИЕ СТУДЕНТОВ К ЭКОЛОГИЧЕСКИМ ПРОБЛЕМАМ В ГРУЗИИ

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Целью исследования явилось определение отношения студентов к проблемам окружающей среды, анализ полу-

ченной информации и разработка мероприятий по участию студентов в природоохранительных оздоровительных и профилактических активностях. Известно, что значимой проблемой в Грузии и во всем мире по сей день является загрязнение воды, почвы, воздуха, напрямую воздействующее на здоровье человека и ухудшающее его. Опрос студентов посредством углубленного интервью выявил, что студенты совершенно не вовлечены в природоохранительную деятельность, их участие в оздоровительных мероприятиях ограничивается только деятельностью в так называемых одноразовых компаниях. На основании полученных результатов рекомендована разработка комплексных мероприятий и схем для стимулирования участия студентов в осуществлении общественно-активных алгоритмов по улучшению состояния окружающей среды.

რეზიუმე

სტუდენტების დამოკიდებულება ეკოლოგიური პრობლემების მიმართ საქართველოში

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მსოფლიოში არსებული გლობალური საფრთხეებიდან გარემოს დაბინძურება ერთ-ერთ უმნიშვნელოვანეს ფაქტორად ითვლება. კვლევის მიზანია სტუდენტების დამოკიდებულების შესწავლა ეკოლოგიური პრობლემების მიმართ. თვისებრივი კვლევის ფარგლებში ჩატარდა უნივერსიტეტის სტუდენტების სიღრმისეული ინტერვიუება. სტუდენტების აზრით, საქართველოში ყველაზე მნიშვნელოვან პრობლემას წარმოადგენს ჰაერის, წყლის რესურსებისა და ნიადაგის დაბინძურება. რესპონდენტები ნაკლებად არიან ჩართულნი გარემოსდაცვით ღონისძიებებში. მიზანშეწონილია შემუშავდეს კომპლექსური ღონისძიებები, რათა მოხდეს სტუდენტების მონაწილეობის სტიმულირება გარემოს დაცვასთან დაკავშირებული პრობლემების მოგვარების საქმეში.

OBLIGATIONS TO INDEMNIFY DAMAGES INFLICTED BY MAIMING AND OTHER PERSONAL INJURIES INCLUDING DEATH: THEORETICAL AND PRACTICAL ISSUES (REVIEW)

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Obligations to indemnify damages inflicted by maiming and other personal injuries including death are not contractual in nature, and regulatory management of such obligations has its roots far back in Roman law. The civilized world puts the highest value on human life and death, hence, inflicting of personal injury is considered to be a breach of absolute rights. Accordingly, the duty to indemnify for damages is imposed on a wrongdoer due to his failure to fulfil an absolute obligation of the passive type in absolute legal relations.

In spite of the fact that most law-governed states place particular emphasis on seeking legislative action to solve issues dealing with indemnification for damages inflicted by maiming and other personal injuries including death, there are a large number of both civil and criminal cases in this field. In particular, numerous difficulties arise in personal injury cases when injury to the patient was caused by the doctor's treatment or the denial of medical care. The healthcare environment is a priori fraught with increased risks of inflicting injuries; moreover, the procedure for establishing medical negligence is complicated and involves the combination of the law and medical science field. The assessment of damages the injured patient is entitled to also presents considerable difficulties.

A set of issues connected with the regulatory management of indemnification for damages inflicted by maiming and other personal injuries including death as well as personal injury arising from the doctor's treatment or the denial of medical care has been studied by a number of scholars including S. Antonov, S. Buletsa, D. Hrymm, S. Hrynko, U. Hryshko, Yu. Danysheva, K. Drishliuk, A. Mamushkina, O. Ruban, O. Chernilevska and others.

The article aims to provide a comprehensive review of the legal basis for and special features of indemnification for damages inflicted by maiming and other personal injuries including death, in particular, due to the doctor's treatment or the denial of medical care. The present research involves critical analysis of civil legislation of Ukraine and practice of European Court. It also employs the legal framework governing out-of-court and in-court settlement of disputes with regard to inflicting personal injury to patients due to the provision of medical assistance or failure to provide medical assistance and some aspects of the assessment of damages to be awarded to the injured patient. In order to identify common trends relating to court decisions on damages in personal injury and wrongful death cases in the healthcare setting, some high-profile cases are considered, in particular 8 decisions made by domestic national courts of Ukraine as well as 17 decisions made by the European Court of Human Rights.

The present research employed the comparative legal research method in order to compare the features of the regulatory management of indemnification for damages inflicted by maiming and other personal injuries including death in Ukraine and other countries. The integrated system-wide approach was used to examine the reasons for placing the liability for inflicting personal injury to the patient on subjects providing medical care. The method of analysis and the inductive method were applied to make generalisations providing useful practical precedents relating to compensation for damages in medical malpractice

cases. The method of modelling was applied to study the content of legal relationship with regard to indemnification for damages inflicted by maiming and other personal injuries including death that is the subject of the court cases under consideration.

In spite of the fact that obligations to indemnify damages inflicted by maiming and other personal injuries including death belong to non-contractual (delictual) obligations, the Civil Code (hereinafter CC) of Ukraine provides for cases when rules that regulate non-contractual relations extend to some particular obligations that arise from contracts. Then, rules of delictual responsibility are applied regardless of whether an act that caused an injury is necessary for the performance of a contract. Specifically, Article 928 of the CC of Ukraine provides that liability for personal injury or death in respect of passengers is delineated according to Chapter 82 of the CC of Ukraine "Indemnification" unless the contract or the law provides for strict liability of the carrier. More specifically, although this refers to the losses connected with the performance of the contract, the rules of delictual responsibility are applied due to the respective statutory reference [5].

In addition, if more than one person causes a personal injury, tortfeasors and the victim may enter into an agreement to ensure joint and several liability. The essential terms of such an agreement shall set a time period for compensation payment, as well as a procedure for and a method of providing indemnity for an injury inflicted jointly by several people. The parties may also agree on the procedure for defining compensatory damages. Although these provisions will duplicate generally recognized civil law regulations, a contract for compensatory damages may contain provisions for liability for non-performance or unsatisfactory performance of contractual obligations [ECHR Case of *Asiye Genç v. Turkey* (Application № 24109/07)].

However, it is understood that in spite of the existence of the contract that provides for the liability of the parties to pay full compensation for a personal injury caused to the other party or the contract that sets the procedure for paying compensatory damages inflicted by more than one person, the obligation itself to indemnify damages is delictual, i.e. non-contractual in nature. These relations always fall under the mandatory provisions of Chapter 82 of the CC of Ukraine. The terms that are provided for by the contract for compensatory damages cannot worsen the situation or limit or diminish any rights of the indemnitee as compared with his rights under the CC of Ukraine.

It should be emphasized that the categories "obligation to indemnify" and "liability for damages" that are often used interchangeably in law books differ in meaning. In research literature, indemnification is proved to be a method of the performance of an obligor's obligation and the fulfilment of his duty rather than a sanction for a tortious act. Reasons for an obligation to indemnify damages are the tortious actions themselves that cause an injury, while reasons for delictual liability is an act of law enforcement. Tortious actions serve only as a reason for rendering a decision on imposing liability (given the right circumstances) but not a reason for liability [15].

Damages inflicted by death and damages inflicted by maiming and other personal injuries are different in content.

If damages are inflicted by the death of a person, the actual loss of the affected individuals is funeral and burial expenses and gravestone installation costs. In this case, the loss of benefit is the amount of maintenance that the survivors specified by Article 1200 of the CC of Ukraine and maintained by the deceased worker received in his lifetime and lost due to the breadwinner's death.

A special feature of the relationship arising from indemnification for damages inflicted by maiming and other personal injuries including death is a possibility for the victim to establish consequential property and non-property damages that may occur even over a significant time interval following the day on which the accident occurred [15].

According to the CC of Ukraine, if damages are inflicted by health injuries, the actual damage of the victim must be considered to be health recovery and/or rehabilitation costs. These costs are associated with the necessity of nourishing diet, health resort treatment, purchase of medicine, prosthetic repair, nursing care, etc. [15]. However, this list is indicative, hence, in cases when the victim has suffered other damages that are connected with the relevant personal injury, he has the right to claim their compensation as well. The Roman jurists used the same approaches by granting the victim the right to claim compensation for treatment and loss of earnings according to *legis Aquiliae utilis* [3].

In addition, the loss of benefit is damages in the form of un-received income which an individual would receive under ordinary conditions had his right to full-time work not been violated. When the amount of income is estimated, an important factor is whether the individual was in work. More specifically, if maiming or other personal injuries was caused to a person who worked under an employment contract, the amount of lost income that is subject to compensation shall be calculated as a percentage of the average monthly income the victim received before being injured taking into account the percentage of loss of both professional and general working capacity or general working capacity if there is no professional working capacity (Section 1 of Article 1197 of the CC of Ukraine).

Should loss of earning capacity be permanent, the victim must be sent for medical examination to estimate the degree to which earning capacity has been lost; should the level of disability be high, the victim may be assigned a disability category and granted a pension by a social safety net. Based on these facts, the amount of caused material damage is estimated. Reimbursement for lost income or its part does not include the following: disability benefit that was granted to the victim due to maiming and other types of pensions that were awarded preceding health injury as well as following it on account of the compensation for damages. Neither does it include income the victim receives after being maimed [2].

Loss of earnings (income) includes all the types of remuneration under an employment contract that are subject to tax in both full-time and part-time positions in the amounts of pretax earnings. However, lost earnings (income) do not include single payments, compensation for unused vacation, severance package, maternity benefits, etc. (Section 3 of Article 1197 of the CC of Ukraine). A similar rule is provided in item 4, Section 13 of resolution of the Plenum of the Supreme Court of Ukraine No 6 dated March 27, 1992 [13].

The CC of Ukraine contains the rule according to which "damage inflicted by maiming and other personal injury should be indemnified regardless of the pension assigned to an individual due to disability or the pension the individual received before the injury or other income" (Section 3 of Article 1195).

This means that the victim is entitled to obtain both: 1) reimbursement in full from the tortfeasor according to delictual obligations; and 2) pensions or aid under social insurance according to the rules of social insurance. Accordingly, a social insurance agency has no right of subrogation against a tortfeasor in respect of benefits paid from the social insurance schemes due to the fact that relations under the right of subrogation are regulated according to the rules of delictual obligations (item 1 of Section 1191 of the CUV) rather than social insurance rules. This approach to the reimbursement procedure is progressive. It was implemented in Ukrainian legislation only following the entry into force of the Civil Code of Ukraine of 2004.

Hence, judicial decision-making in respect of awarding damages inflicted by maiming or other personal injuries should be driven by the fact that the victim is entitled to reimbursement of damages inflicted by maiming or other personal injuries in full according to Article 1166 of the CC of Ukraine. First, estimating the amount of damages involves both actual damage and loss of benefit. Second, defining the types of expenses that are subject to reimbursement should ensure that these expenses will make the victim's recovery to the fullest extent possible. Moreover, Yu. Danysheva argues, compensation for additional costs should be provided with respect to the optimal medical treatment without limiting reimbursement to a particular amount [5].

When dealing with the extent of compensation for damages, it is necessary to emphasize that there is a distinct difference between the notions *extent of compensation for damages* and *amount of compensation for damages* inflicted by maiming or other personal injuries including death. Article 1166 of the CC of Ukraine sets forth the basic principle of the civil law according to which damage caused to the victim is subject to indemnification in full, in other words, the victim is entitled to indemnification for all the types of possible damages caused to him including both property damage and non-property damage. In this regard, A. Mamushkina assumes that the extent of compensation is a value that encompasses the types of the damage that can be caused to an individual. While the amount of compensation for damages is closely related to the extent of compensation, they do not coincide because the former must be estimated in monetary or other terms, thus being a quantitative description of inflicted damages [8].

Pursuant to the general rule, any act that causes personal injury is assumed to be illegal. A causal connection between an illegal act and inflicted damage has specific features. In regard to these delictual obligations, it is complicated in nature, which is due to the specific character of inflicted damage. In other words, it is necessary to prove that there is a causal connection between illegal acts and an inflicted personal injury as well as a connection between the personal injury and incurred material expenses. However, it should be noted that in Ukraine there are judicial precedents for compensation for damages due to an indirect causal connection. For example, a technically imperfect surgical procedure (which resulted in leaving a retained foreign body inside the patient's body) necessitated repeated surgery to retrieve the foreign body. However, the medical expert review points out that this was not the only cause of the development of postsurgical complications in the victim, hence, it should be plausible to suggest that there is an indirect causal connection between them and the deterioration of the victim's health condition. Nevertheless, the victim was awarded 20 000 UAH (833\$) compensation for moral damages [Рішення Шевченківського районного суду м. Києва від 24 червня 2016 року. Case № 2610/25254/2012].

The Roman legal tradition is also manifest in the fact that the obligation to indemnify for damages inflicted by a personal injury arises in case a tortfeasor is at fault (Section 1 of Article 1166 of the CC of Ukraine). In this regard, the form of fault is of no importance for the creation of obligation. That is why the Roman jurist Paul's maxim is still relevant today: «With respect to a lawsuit, under the *lex Aquilia*, malice and gross negligence are punishable» [4].

Similarly to general rules, the tortfeasor's fault under this delict is presumed until the tortfeasor proves that he has no fault in it. However, in cases provided for by law, a personal injury is indemnified regardless of the tortfeasor's fault (Article 1173–1176, 1187 of the CC of Ukraine). In some cases that are directly stipulated for by law, damages inflicted by maiming or other personal injuries including death due to force majeure also may be subject to indemnification (Section 3 of Article 1166 of the CC of Ukraine from 16.01.2003, № 435-IV).

In judicial practice, a special procedure for indemnification for damages has been established for cases relating to personal injuries inflicted to individuals under the age of majority (minors). This is due to the fact that compensation for damage inflicted by a minor's injury in respect of its part connected with the loss of earnings is impossible to estimate according to the general criteria. This is why special rules for estimating the amount of inflicted damages have been elaborated.

Under the CC of Ukraine, minor victims are entitled to indemnification for damages due to the loss or diminution of future earning capacity upon reaching the age of fourteen (students upon reaching the age of eighteen) based on the minimum salary set by law. If by the time of injury, a minor had an income, damages should be indemnified based on his earnings but not less than the minimum salary set by law (Article 1199).

There is also a special procedure for indemnification for damages caused by a criminal offence. Under Article 1207 of the CC of Ukraine, damages inflicted by maiming or other personal injuries including death due to a criminal offence are indemnified to the victim or other individuals who are entitled for indemnification by the state unless an individual who committed a crime is identified or is solvent. The terms and procedure for indemnification for damages inflicted by maiming or other personal injuries including death by the state are established by law. However, at present, there is no special law which regulates the terms and the procedure for such indemnification. This is why filing a suit and exercising the right to indemnification for damages by the state is impossible. Legislative inefficiency of the rule provided for by Article 1207 of the CC of Ukraine is unacceptable with regard to a difficult economic situation of Ukraine. The procedures implemented in the USA and Great Britain, in particular, indemnification rates set by law, the mechanism for fund raising, etc., may serve as examples of the development of compensation mechanisms in the criminal process [6].

Damages inflicted by a personal injury are indemnified in monthly instalments (Article 1202 of the CC of Ukraine). This is due to the fact that the person who must compensate for damages obtains his income predominantly on a monthly basis. In civil law, there is a precedent for another regime for making compensatory payment. Specifically, under the Code of civil laws of the Russian Empire of 1832 the tortfeasor was obliged to make an annual payment in the amount set by court (Article 661) [4].

Providing there are reasonable grounds and taking into consideration the tortfeasor's financial situation, compensation may be paid in one lump sum but not more than for three years in advance (item 2 of Section 1 of Article 1202 of the CC of Ukraine).

The court may also take into account the defendant's concern to make a lump sum payment and determine the defendant's intention with regard to obtaining a one-time compensation payment. In certain circumstances, like those involving the recovery of additional costs, such a payment may be made in advance within time limits imposed on the basis of a relevant medical expertise report as well as in order to pay bills or for property (health resort package, transport fares and special transport expenses, etc.) if advance payment is required (Section 2 of Article 1202 of the CC of Ukraine).

Therefore, a special feature of obligations to indemnify for damages inflicted by personal injuries including death relates to the object of offence and harmful effects. The object of offence is non-property rights and benefits, hence, it is non-property damage having no cost value that is caused to an individual. However, this offence involves the emergence of additional costs incurred by the victim or the loss of any material benefits and perks, hence, compensation for non-property damage acquires material attributes. Therefore, cases involving personal injuries including death may be associated with the possibility of the existence of non-property damage connected with property. A similar approach was adopted by Roman jurists who made decisions on cases on indemnification for damages inflicted by personal injuries including death according to the rules for indemnification for damages caused to property associated with the delicts of *furtum* and *damnum iniuria datum*. Hence, both in Ukraine and in other countries of the Commonwealth of Independent States, rules for indemnification for damages inflicted by personal injuries including death have been developed under the influence of the Roman private law [4].

Particular attention should be paid to the issue of indemnification for damages inflicted by maiming or other personal injuries including death due to medical malpractice. When seeking medical advice, an individual expects to be provided with high quality medical care to be able to make a quick recovery. However, it is often the case that medical or healthcare professionals make a medical error, thus, causing harm to the patient's health, which can even result in the patient's death. In such cases, the patient or his relatives try to seek justice in court. Yet, in Ukraine, very few cases are decided in patients' favour because it is very difficult to prove a medical error or medical negligence. Litigation in such cases can last for several years since investigation into the negative effects on the patients' health or causes of his death involves special knowledge in medicine, which means assigning a forensic medical examination, or more than one examination if experts' views do not coincide or there are still disputable issues to settle for the parties.

The fact that medical malpractice cases are a common category of cases heard by the European Court of Human Rights (hereinafter ECHR) is yet more proof of its complex nature. Although the right to health does not belong to the rights that are guaranteed by the Convention for the Protection of Human Rights and Fundamental Freedoms of 1950 (hereinafter the Convention), the issues dealing with the civil liability for damages inflicted by maiming or other personal injuries including death that were caused by a medical or healthcare professional are considered under Articles 2, 3 and 8 of the Convention, which means these issues are regarded as the violation of the right to life, prohibition of torture and the right to respect for private and family life.

For example, the ECHR in its judgments has pointed out a number of times that in respect of medical negligence it is necessary that there should be proper procedural tools for bringing a medical or healthcare professional to criminal, civil or

disciplinary liability in order to ensure the exercise of fundamental human rights (in particular, patients' rights) [ECHR Case of Mehmet Günay et Güllü Günay v. Turkey (Application № 52797/08)]. Regardless of the type of trial, litigation should involve all significant elements in order to shed light on the circumstances involved in the case [Case of Asiye Genç v. Turkey (Application № 24109/07)].

However, with regard to medical negligence, in order to ensure the proper protection of the patient's right it may be sufficient to provide a possibility to appeal against medical negligence in a civil court and impose any relevant civil sanctions such as indemnification for damages [ECHR Case of Benderskiy v. Ukraine (Application № 22750/02)].

Under Article 1195 of the CC of Ukraine, indemnification for damages inflicted by maiming or other personal injuries including death is provided by a legal body (a medical facility) or a physical person (a physician who practices medicine under a license). It should be noted that the patient is entitled to compensation for his lost earnings (income) due to loss or diminution of professional or general working capacity as well as additional expenses incurred by the need for a high calorie diet, health resort treatment, purchase of medicine, prosthetic repair, nursing care, etc. Under Article 1172 of the CC of Ukraine a legal body or a physical person indemnifies damages caused by their employee during the course of the performance of his employment duties, which means that compensation for damages will be withdrawn from the employer (a medical facility or a private entrepreneur if damage was caused by an individual employed by him). In case damage was caused by the entrepreneur himself who has a license for medical practice, the means will be withdrawn from him. In addition, the patient is entitled to indemnification for moral damage under Article 23 of the CC of Ukraine. This damage consists of both physical pain the individual suffered from due to incorrect treatment in hospital and physical pain that resulted from improper medical care as well as pain caused while "correcting" the medical error.

In addition, moral damage involves emotional suffering an individual experiences due to the physicians' actions that is manifested by health anxiety, concerns that prescribed treatment does not work, etc. However, one of the complexities associated with patients who suffered from doctor's negligent treatment is the necessity to prove the causal connection between doctor's actions and an inflicted personal injury. Most often, this is done by assigning a forensic medical examination. In this category of cases, the expert opinion is given *de facto* high priority as evidence. At this stage, litigation often involves the so-called medical ethics which is nothing more than a cover-up. As a result, it is often not possible for a forensic medical examination to evaluate doctor's actions / negligence with regard to causing harm to the patient. In the end, it is often the case that the patient doesn't receive any indemnification for damages due to the dismissal of a case on the ground that there is no evidence of causal connection between doctor's actions / negligence and effects.

Legal remedies for the violation of personal non-property rights of patients in the course of and due to receiving medical care include the right to indemnification for damages as well as other methods of reimbursement for material damage. In the healthcare sector, the liable party is healthcare facilities as well as medical and healthcare professionals who run a private medical practice. Civil liability is divided in two types:

- 1) contractual; and
- 2) delictual [1].

Contractual and delictual liabilities are differentiated by the

rule according to which should there be reasons for contractual remedies, non-contractual remedies are dismissed: contractual remedies require pre-emption of non-contractual ones. This, of course, does not mitigate liability established by law. A decision on contractual liability is made provided the parties have a contractual obligation. If there is no contractual obligation, it is acceptable to consider imposing delictual liability. If medical care is provided based on a repayable contract, contractual liability is invoked. In this case, its extent cannot be less than that of delictual liability. On the contrary, the level of compensation for damage may be raised as compared to the general rules. In such cases, the role of the contract consists in the fact that through the contract an obligation to indemnify for damages stipulated for by law can be realised with respect to: reasons for an obligation; parties involved; and its content. While looking into the issues connected with contractual obligations, it should be noted that, under section 2 of Article 901 of the CC of Ukraine, the rules of Chapter 63 of the CC of Ukraine "Services. General provisions" may be applied to all service supply agreements (including medical services) provided this does not contradict the essence of the obligation. Civil legislation of Ukraine involves the principle of presumed guilt of the tortfeasor in the course of the provision of medical care, which puts the burden of proving innocent on the tortfeasor. Under the general rule that is provided for by Section 2 of Article 1166 and Section 1 of Article 1167 of the CC of Ukraine, the tortfeasor is exempted from the obligation to indemnify for property and moral damages if he proves that the damage did not occur through his fault.

In the healthcare sector, property damage consists of:

a) actual damage that is loss an individual incurred or has to incur in order to restore the right that has been infringed (costs associated with drug purchase, prosthetic repair, the performance of diagnostic tests, treatment and rehabilitation, health resort treatment, nourishing diet, nursing care, funeral and burial, and gravestone installation (Item 1 of section 2 of Articles 22, 1195 and 1201 of the CC of Ukraine)); and

b) loss of benefit that is income an individual could have received under ordinary conditions had his right not been violated (lost earnings (income) due to loss or diminution of professional or general working capacity (Item 2 of section 2 of Articles 22 and 1195 of the CC of Ukraine from 16.01.2003 p. № 435-IV).

The extent to which professional working capacity was lost (expressed as percentage) and the need for additional types of assistance are determined by forensic medical examination. In personal injury cases, should additional costs arise due to the need for health and social care, they must be indemnified by the tortfeasor. Moreover, the need for such costs and their duration must be proven by the conclusion of forensic medical examination. Additional costs may be charged in advance within a time period set forth in the conclusion of the disability determination agency or forensic medical examination. When charging costs associated with prosthetic repair and health resort package, the court must indicate in the decision that awarded amounts are liable for transfer to a relevant organization which must provide the victim with these services. In the event of the victim's death, the tortfeasor (either an organization or an individual) are entitled to indemnify funeral and burial expenses (including ceremonial services and funeral arrangements) to the individual that incurred these expenses. This is why a claim for funeral and burial expenses may be filed by individuals who are entitled to indemnification for damages due to the breadwinner's death as well as individuals and organizations who are not related to the victim, yet, actually incurred these expenses. Gravestone

and gravestone fences installation costs are determined based on their actual cost, but not higher than the upper limit cost of standard gravestones and fences in the location in question [13].

Any legal remedy must be effective in practice in respect of the protection of the rights of an individual (including a patient) [ECHR Case of Calvelli and Ciglo v. Italy (Application № 32967/96)]. Specifically, indemnification for damages seems to be simple and easy in theory. But how does this work in practice and how can medical malpractice be proven?

First, it is necessary to prove medical negligence which consisted in misdiagnosing the patient's condition or administering the wrong treatment. The guidelines for healthcare delivery were approved by the order of the Ministry of Healthcare of Ukraine. They set forth which tests and diagnostic procedures must be performed to confirm a primary diagnosis and make a decision on treatment. Specifically, order No 602 dated August 3, 2012, provides for the execution of medico-technological documentation with regard to the standardization of healthcare procedures for stroke [9].

These standards are set forth in medico-technological documents which were developed according to the multi(inter-)disciplinary (hereinafter multidisciplinary) approach to a specific issue, i.e. a medical issue (a disease or another pathological condition, living a healthy lifestyle, etc.) rather than a medical specialty [14]. This is why before taking legal action it is necessary to identify what the patient was diagnosed with in a healthcare facility. Under Articles 34 and 49 of the Constitution, Article 285 of the CC of Ukraine and Article 39 of the Fundamentals of the Legislation of Ukraine on Healthcare, the patient has the right to request any document that is available in a healthcare facility (in particular, a patient's chart, all test results and a treatment card. For example, mandatory diagnostic tests and procedures for ischaemic stroke are as follows:

- brain imaging which is performed in patients with acute cerebrovascular accident (ACA) as a matter of priority (within 24 following the onset of the disease);
- laboratory and instrumental diagnostic tests which are performed in order to clarify a patient's clinical picture, assign treatment, and identify the type of stroke to assign stroke secondary prevention services (a detailed classification of stroke types is given in adapted clinical guidelines);
- patients with stroke and transitory ischaemic attack (TIA) are recommended to be scheduled 24-hour Holter monitoring to check their electrocardiogram (ECG) following the acute period provided the patient has arrhythmia and stroke type has not been identified.

The following treatment is assigned: 1) provided the possibility of haemorrhagic stroke has been eliminated based on brain imaging, within 48 hours following the disease onset patients with ischaemic stroke must be administered acetylsalicylic acid (ASA) with a dose of 160–325 mg (for patients with no dysphagia per os, and for patients with dysphagia into the nasogastric tube, intramuscularly or per rectum); 2) ASA therapy with the dose indicated should be continued until two weeks when the dose can be reduced to preventive (usually 75–100 mg/day). Instead of ASA, for the purpose of secondary prevention other clot-busting drugs can be prescribed (Clopidogrel or a combination of aspirin plus extended-release dipyridamole); 3) patients with TIA must be administered 160–325 mg/day as soon as possible provided there are no contraindications.

Quality criteria (expected treatment outcomes): 1. The length of in-hospital treatment depends on the level of stroke severity and is from 7 to 28 days (of them 7–14 days in a specialist stroke

unit). 2. Early discharge of the patient from a stroke care unit is possible if the level of stroke severity is either minor or moderate and providing the rehabilitation recommended by a multidisciplinary team is continued in a healthcare facility which provides outpatient secondary healthcare. 3. After discharge, patients must be under constant surveillance of a neurologist in a healthcare facility which provides outpatient secondary healthcare and a general practitioner in their home area to continue following the prescriptions of the multidisciplinary team [9].

When the claimant obtains all the documents, he can compare the diagnosis and assigned treatment with the medical standard of care that establishes the doctor's mandatory conduct. Only in case the claimant identifies any irregularities, it can be said that doctors failed to provide competent care and made diagnostic error and/or tactic error (for example, when a diagnosis is inappropriately delayed, which resulted in the development of a disease [ECHR Case of Lopes de Sousa Fernandes v. Portugal (Application № 56080/13)]).

However, when it is impossible to identify the negative consequences for the patient's health and life, or there is a probability that a chosen method of treatment will have a negative impact (for example, due to medication administered to the patient), there are no reasons for indemnification for material and moral damages [ECHR Case of Mehmet Günay et Güllü Günay v. Turkey (Application № 52797/08)].

Under the Constitution of Ukraine everyone has the right to health care (Article 49) [Constitution of Ukraine from 20.06.1996 p. № 254к/96-BP], which the state realizes through healthcare facilities. In such a case, a failure to provide medical treatment or denial of medical care by medical and healthcare professionals, who are aware of the health and life risks an individual faces, may be a reason for compensation for damages [ECHR Case of Elena Cojocaru v. Romania (Application № 74114/12)]. Based on the practice of ECHR, it should be noted that, in particular, the reasons may be as follows: denial to provide proper medical care and transfer to another hospital owing to the patient's inability to pay medical fees [ECHR Case of Mehmet Sentürk and Bekir Sentürk v. Turkey (Application № 13423/09)]; failure to provide the patient with expensive anti-cancerous medication for free in spite of the fact that under the current legislation he is entitled to it [ECHR Case of Panaitescu v. Romania (Application № 30909/06)]; doctor's refusal to terminate pregnancy despite the certificate issued by a general practitioner stating that the pregnancy constituted a threat to the patient's health [ECHR Case of Tysięc v. Poland (Application № 5410/03)].

Second, prove the fact of a resulting injury to the patient. Having obtained hard evidence that diagnostic tests or treatment assigned by the doctor deviated from the accepted medical standard of care, the applicant (claimant) must prove the fact of a resulting injury to the patient. The injury may be proven by the following: facts of consulting other doctors (who assigned treatment); facts of disclosing other diseases or the development of a chronic form of the disease, which might have resulted from improper treatment; written opinions of other doctors who examined or treated the patient provided the opinions have the effect of expert medical report. For example, order of the Ministry of Healthcare of Ukraine № 110 of 14 February 2012 "On approval of primary medical record documentation which is used in healthcare facilities, public or private, and instructions on recording information" approved form № 043/o for dental patients "Dental patient record card" [10]. According to the Instruction on recording information in primary medical record documenta-

tion № 043/o “Dental patient record card”, the patient record card contains an entry “Complaints”. This entry should record complaints stated in patient’s or his relatives’ words that describe the patient’s condition in the most accurate way. A special section of the patient record card is designed for planning dental examination of the patient and treatment of the patient with specialist consultation records. The section of the patient record card under the title “Diary” is designed for recording subsequent patient encounters in connection with the given condition or any other conditions. It is followed by the epicrisis which contains a brief description of treatment outcomes and practical procedures recommended by the doctor. After the treatment finishes, the patient record card is signed by both the doctor who administered the treatment and the head of the department, while after each particular stage of treatment finishes it is signed by the doctor who is actually treating the patient [7]. In case any of the sections of the patient record card is missing, it can be assumed that doctors failed to fill it in in an accurate way owing to their negligent attitude towards their duties or that part of the medical record documentation was altered to cover up medical malpractice.

Third, it is necessary to establish a causal connection between medical or health care professional’s actions and the patient’s resulting injury. Specifically, a failure to establish a causal connection between medical or health care professional’s actions and an injury caused to the patient results in the violation of the right to a fair trial (Article 6 of the Convention) and fair hearing rather than the right to physical integrity as an element of the right to respect for private and family life protected by Article 8 of the Convention [ECHR Case of Benderskiy v. Ukraine (Application № 22750/02)].

In this case, the claimant must prove that it was doctor’s action that caused harm to the patient rather than other objective reasons such as medical care as an imperfect science at its present stage, objective diagnostic challenges, atypical clinical course and consequences, late diagnosis caused by patient’s late presentation, etc. For example, in the case of Trocellier v. France the victim claimed damages inflicted by an injury to her health based on the fact that the doctors failed to inform her about a risk of negative consequences of the surgery. The expert report confirmed that the paralysis occurred immediately after the operation and disappeared within three months. However, the symptoms of paralysis that the patient experienced were of a psychosomatic nature. The ECHR pointed out that a body of medical research into psychosomatic symptoms had been scarce and that the data available were still based on hypothesis, which had made it difficult to include them as a matter of principle while providing the information to patients. Due to this, the victim was declared to have no reasons for getting the compensation for damages [ECHR Case of Trocellier v. France (Application № 75725/01)]. An imperfect regulatory framework that governs medical practice and may cause medical malpractice [ECHR Case of Arskaya v. Ukraine (Application № 45076/05)] and irreparable harm constitutes a completely different situation. For example, in the case *Asiye Genç v. Turkey*, a causal connection between the death of the new-born baby and the facts that there was a lack of space in the neonatal intensive care unit and the baby had to be transferred to another hospital to subsequently die in the ambulance was proven by the forensic medical examination on the national level. In its judgment, the ECHR held that the baby was the victim of the improper functioning of the public hospital service. A situation is different when doctors admit that there are shortcomings (for example, a lack of

necessary equipment) in a healthcare facility. In this case, resulting consequences will result from both medical malpractice and structural shortcomings of a healthcare facility that in their combination made the delivery of urgent medical care impossible, which is equivalent to the denial of medical care resulting in putting life at risk [ECHR Case of Aydođdu v. Turkey (Application № 40448/06)]. A lack of coordination between medical facilities or shortage of medical staff in a particular hospital unit [36] may be yet more proof of improper functioning of health care providers.

Although a physician must apply his skills to save life and act in a patient’s best interests [ECHR Case of Arskaya v. Ukraine (Application № 45076/05)], with such factors available, it is unreasonable and against the principle of justice to put the blame for negative consequences on the physician.

Third party fault should be taken into consideration too. For example, the patient sought medical advice in another hospital or applied to alternative medicine or practiced self-treatment. However, home delivery may be considered an exception. For example, even provided home delivery is allowed by law, it is inadmissible to refuse to provide medical care because under no circumstances a baby should be devoid of the right to medical care based on the fact that it was born out of hospital [ECHR Case of Kosaitė-Čypienė and others v. Lithuania (Application № 69489/12)].

If an individual is certain to be able to prove all the above mentioned elements, he can file a lawsuit. However, it must be borne in mind that under item 7 of Section 1 of Article 1 of law of Ukraine of 01 December 2005 No 3161-IV “On Consumer rights protection”, a contract is a juristic act, either oral or written, between the consumer and the seller (performer) about the quality, time period, price and other terms under which products are managed. An oral contract is performed by executing a receipt, a sales or cash voucher, a ticket, a coupon or any other documents (hereinafter payment document) [14]. Hence, when seeking medical advice, an individual enters into an oral contract with a hospital for medical services, thus, being their consumer. This is why the relationships that arise from such a contract are subject to the law “On Consumer rights protection”. In this respect, the consumer’s right to information on goods should also be considered (Article 15 of the law). In the healthcare sector, it is important that individuals whose health has been put at risk (even if these have been singular cases) should have access to information which allows taking an unbiased look at risks, since if the anticipated risk of this kind materializes without the patient being duly informed in advance, the right to physical integrity as an element of the right to respect for private and family life protected by Article 8 of the Convention may be violated [ECHR Case of Trocellier v. France (Application № 75725/01)].

Yet, legal action is not the only way to settle a dispute in a positive way. The individual concerned may present his demands in writing as a complaint to healthcare facilities. A response to the complaint must be given in writing within the timeframe established by the law of Ukraine “On citizens’ appeals” [12]. Then, in case of failure to settle a dispute in such a way, the response to the complaint will serve as evidence in court. If the demands are rejected by a healthcare facility, the complaint should be lodged to the executive authorities which ensure the realization of the national policy in the healthcare sector. In case of failure to settle a dispute without resort to court, a lawsuit should be filed. Sometimes, it is easier to prove a medical error in a criminal case, especially if doctors’ actions caused grave consequences for the patient such as the patient’s death, suicide or personal

injuries (grave or medium grave injuries). Although with regard to medical malpractice the possibility of taking proceedings for civil liability is sufficient in principle [Case of Yardimci v. Turkey (Application № 25266/05)] and characterizes the state as ruled by law [ECHR Case of Trocellier v. France (Application № 75725/01)], in exceptional circumstances when the fault, which is assigned to a healthcare provider, is far beyond a medical error or negligence the mechanism for criminal prosecution is necessary [ECHR Case of Vlase v. Romania (Application № 80784/13)]. Specifically, Article 140 of the Criminal Code of Ukraine establishes doctors' liability for failure to perform or improper performance of their professional duties owing to negligent or unconscientious attitude to them providing this caused grave consequences for a patient [Criminal Code of Ukraine from 05.04.2001 p. № 2341-III]. In this case, it is reasonable to appeal to law-enforcing bodies and after proving doctor's fault by the court bringing in the verdict of guilty to file a lawsuit within the framework of a civil case. In these circumstances, the facts a claimant refers to will have been proven in a criminal case, thus, there will be no need to prove them. However, if a relative of a deceased patient accepts compensation within a civil medical negligence case, he cannot act as a victim in other proceedings, in particular criminal proceedings [ECHR Case of Powell v. the United Kingdom (Application № 45305/99)].

It should be noted that it is impossible to prove negative consequences for the patient's health or establish causes of the patient's death without special knowledge in medicine, which means assigning a forensic medical examination or more than one examination in case experts have different opinions or the parties still have unsettled issues. Provided the expert conclusion is deemed incomplete or ambiguous, the court may assign additional expertise to be provided by the same or another expert (or other experts). This is due to the fact that impartiality of expert opinions in such cases may be automatically challenged since experts are practicing doctors who have ethical duty not to criticize their colleagues [Case of Vasileva v. Bulgaria (Application № 23796/10)]. However, if the expert opinion was deemed groundless or such that contradicts the other materials in the proceedings or causes doubt with regard to its accuracy, according Section 2 of Article 113 of the Civil Procedure Code of Ukraine from 03.10.2017, № 2147-VIII, the court may assign repeated expertise to be provided by another expert (other experts).

The evidence from judicial proceedings shows, that the court considers carrying out an expertise necessary. To illustrate the point, let us consider the decision in case №463/3653/14-ц which granted a compensation claim for moral and material damages to a regional clinical hospital in part and awarded 160 000 UAH compensation for moral damages to claimants (parents of a deceased baby). The claimant gave birth to the baby by caesarean section which was performed by an operating surgeon. The baby was a full-term newborn, postnatal condition was satisfactory. However, within 15 minutes after the birth, the baby's condition deteriorated and the baby was transferred to the neonatal intensive care unit where it died of encephalopathy caused by traumatic brain injury. According to the conclusion of forensic medical examination by the panel of doctors, the cause of death of the newborn baby was a birth brain injury in the form of massive brain haemorrhage accompanied by cerebral blood flow failure with secondary permanent brain damage and impaired function of the vitals. The cause of death was confirmed by the autopsy and pathohistological examination.

The court noted that under Section 1 of Article 1172 of the CC of Ukraine a legal person shall indemnify for the damages

caused by their employee during the course of the performance of his employment duties and the claimants had suffered damage caused by the employee's illegal actions during the course of the performance of his employment duties. Hence, the court held that the damage was subject to compensation by the regional clinical hospital.

In respect of the amount of compensation for moral (non-property) damage, the court determines it depending on the nature and extent of sufferings (physical, emotional, psychological, etc.) claimants experienced, the nature of non-property losses (its duration, recovery capability, etc.) and other circumstances. In particular, the following factors are considered: the victim's health condition, seriousness of forced changes in his life and working circumstances, extent of prestige and reputation loss, time and efforts that are necessary to require to the best of the victim's abilities.

In addition, the court must use sound judgement and bear the hallmarks of equity and reasonableness in its proceedings. When determining the amount of compensation for moral (non-property) damage, the court must provide the relevant motives in its judgment. For example, the court found that in connection with the death of the newborn baby caused by medical negligence the claimants suffered moral damages that manifested themselves as moral pain, caused by the fact that the claimants had been looking forward to the baby's birth but after its death they experienced constant anxiety, worries about their future and the grief of losing a child. Taking into account the nature and extent of the sufferings the claimants experienced, their length of more than four years, the depth of moral pain and impossibility to restore things to the previous state regardless of time and efforts as well as the fact that the baby's mother suffered moral pain caused by an injury to her health, the court held that the defendant was to pay the mother of the deceased baby 100 000 UAH in respect of moral damages and pay the father 60 000 UAH [Рішення Личаківського районного суду м. Львова від 23.02.2015 р., Case № 463/3653/14-ц].

In another case, the extent of moral pain caused by medical negligence which resulted in giving birth to a group I disabled child (the causal connection between the medical negligence and disability was confirmed by the forensic medical examination) with a length of 11 years was assessed by the court to be equal to 4 807 296 UAH [Рішення Красногвардійського районного суду м. Дніпропетровська від 01.04.2019 р. Case № 204/5773/14-ц].

Focusing attention on the judicial proceedings of Ukraine with respect to this category of cases, it should be noted that, apart from the fact that cases when claims have been satisfied are rare, the compensation amount can also be challenged. For example, the court awards compensation for the damage caused to the patient's health by a medical or healthcare professional in the amounts of 60 000 UAH (2 000 Euros) [Рішення Дзержинського районного суду м. Кривого Рогу Дніпропетровської області від 27.04.2011 р. Case № 2-14/11], 20 000 UAH (860 Euros) [Рішення Шахтарського міськрайонного суду від 18.02.2011 р. Case № 2-1/11], 20 000 UAH (690 Euros) [Рішення Шевченківського районного суду м. Києва від 24 червня 2016 року. Case № 2610/25254/2012], 10 000 UAH (345 Euros) [Рішення Ленінського районного суду м. Луганська від 21.12.2007 р. Case № 2-658-2007], 2 000 UAH (69 Euros) [Рішення Красногвардійського районного суду м. Дніпропетровська від 01.04.2019 р. Case № 204/5773/14-ц].

In addition, there is also a precedent for moral compensation

in the amount of 1 UAH. Specifically, apart from non-property claims (the adjudication of acts illegal and the protection and restoration of rights) the victim claimed and was awarded 1 (one) UAH moral compensation from the hospital for failure to provide him with medical assistance and untimely forced discharge of the victim from the hospital without administering the necessary treatment (which caused grave consequences to the victim's health such as total vision loss (the causal connection between the indicated circumstances was confirmed by the forensic medical examination)) [Рішення Вінницького міського суду Вінницької області від 20.05.2016 р. Case № 212/2-4174/11].

It should also be noted that the realization of guarantees with regard to the protection of victims against medical negligence also involves considering contradictions that may arise, in particular, a risk of unjustifiably imposing liability on medical and healthcare professionals, which can cause harm to their professional morale and encourage them to provide medical practice to the bad of patients, i.e. the so called "defensive medicine" [ECHR Case of Vasileva v. Bulgaria (Application № 23796/10)].

Conclusions.

Hence, summing up everything that is mentioned above the following conclusions can be drawn:

1) liability of a healthcare facility or a private doctor for causing a personal injury to a patient emerges under a contract about providing medical assistance which can be concluded both in oral and written forms;

2) a reason for compensation for material and moral damages inflicted by maiming and other personal injuries including death of a patient is the whole of wrongful act (an act or a failure to act) attributed to a medical or healthcare professional, and consequences in the form of material and/or moral damages inflicted to a patient as well as the causal connection between doctor's wrong-doing and damage caused to a patient;

3) Wrong-doing of a medical or healthcare professional is failure to meet or deviation from the medical standard of care which is established in the relative regulations. Moreover, an act by which damage was caused to a patient might be an intentional act or negligence;

4) Crime of omission committed by a medical or healthcare professional is denial of medical assistance without good reason, for example: failure to provide medical assistance or transfer to another hospital due to incapability of the patient to pay bills; failure to provide the patient with expensive anti-cancerous medication for free in spite of the fact that under the current legislation he is entitled to it; doctor's refusal to terminate pregnancy despite the certificate issued by a general practitioner stating that the pregnancy constituted a threat to the patient's health, etc.

5) the causal connection between acts or failure to act of a medical or healthcare professional and consequences in the form of inflicting maiming or other personal injuries is determined based on a careful study of medical documentation related to a particular patient as well as conducting forensic medical examination. Reasons for compensation for damages caused by medical malpractice should be determined based on direct and indirect causal connection;

6) during hearing a medical malpractice case, a failure to establish the causal connection between doctor's acts/failure to act and damage inflicted to a patient by the national courts leads to the violation of the right to a fair trial and fair hearing rather than the right to physical integrity as an element of the right to respect for private and family life protected (Article 6 of the Convention).

7) in particular cases, medical negligence as a well as other forms of causing personal injuries due to the provision or failure to provide medical assistance must involve both civil and criminal liability;

8) due to the fact that the amount of compensation awarded by court is not often proportionate to caused damage, it is considered reasonable that a compensation rate system be introduced in Ukraine to assess the amount of compensation for damages inflicted by maiming or other personal injuries including death owing to medical malpractice. This system should take into account the special nature of inflicting of damages in medicine, in particular, the nature of doctor's wrongdoing and negative consequences (both material and moral) the patient has experienced or members of his family or relatives in the event of his death.

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SUMMARY

OBLIGATIONS TO INDEMNIFY DAMAGES INFLICTED BY MAIMING AND OTHER PERSONAL INJURIES INCLUDING DEATH: THEORETICAL AND PRACTICAL ISSUES (REVIEW)

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The present article aims to provide a comprehensive review of the legal basis for and special features of indemnification for damages inflicted by maiming and other personal injuries including death, in particular, due to the doctor's treatment or the denial of medical care.

The fulfilment of the aim involved critical analysis of civil legislation of Ukraine, with regard to indemnification for damages inflicted by maiming and other personal injuries including death. It also employs the legal framework governing out-of-court and in-court settlement of disputes with regard to inflicting personal injury to patients due to the provision of medical assistance or failure to provide medical assistance and some aspects of the assessment of damages to be awarded to the injured patient. In order to identify common trends relating to court decisions on damages in personal injury and wrongful death cases in the healthcare setting, 8 decisions made by domestic national courts of Ukraine as well as 17 decisions made by the European Court of Human Rights were considered. The present research employed the comparative legal research method, the integrated system-wide approach, the method of, the inductive method, the method of modelling, etc.

Based on the conducted research, both out-of-court and in-court ways of the settlement of disputes with regard to inflicting personal injury to patients due to the provision of medical assistance or failure to provide medical assistance were identified as well as and some aspects of the assessment of the amount of compensation for damages to be awarded to the injured patient. The article provides a critical description of the reasons for liability of healthcare facilities or private doctors for causing

maiming or other personal injuries including death to patients as well as the special features of this liability in criminal proceedings. It also outlines common tendencies of making decisions on personal injury cases involving the healthcare sector by the European Court of Human Rights. The article examines the role and special features of forensic medical examination as a sound basis for determining the fact of causing a personal injury.

Keywords: damage, health, medical care, European Court of Human Rights, indemnification, treatment.

РЕЗЮМЕ

ОБЯЗАТЕЛЬСТВА ПО ВОЗМЕЩЕНИЮ ВРЕДА, ПРИЧИНЕННОГО УВЕЧЬЕМ, ДРУГИМ ПОВРЕЖДЕНИЕМ ЗДОРОВЬЯ ИЛИ СМЕРТЬЮ: ТЕОРЕТИЧЕСКИЕ И ПРАКТИЧЕСКИЕ АСПЕКТЫ (ОБЗОР)

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Целью статьи является комплексный анализ правовых оснований и особенностей возмещения вреда, причиненного увечьем, другим повреждением здоровья или смертью вследствие предоставления или безосновательного отказа в предоставлении медицинских услуг.

Проведено комплексное исследование гражданского законодательства Украины по вопросам, регулирующим возмещение вреда, причиненного увечьем, другим повреждением здоровья или смертью, а также нормативной базы Украины по регламентации внесудебных и судебных способов урегулирования споров о причинении вреда жизни или здоровью пациентов в результате предоставления или не предоставления медицинских услуг, отдельных аспектов определения размера возмещения за такой ущерб. С целью обобщения судебной практики в части рассмотрения дел о возмещении вреда, причиненного увечьем, другим повреждением здоровья или смертью в сфере оказания медицинских услуг, проанализированы 8 решений, вынесенных внутренними национальными судами Украины, а также 17 решений Европейского суда по правам человека. При проведении исследования использован сравнительно-правовой, системно-комплексный метод, методы анализа, индукции и моделирования.

На основании проведенного исследования определены внесудебные и судебные способы урегулирования споров о причинении вреда жизни или здоровью пациентов в результате предоставления или не предоставления медицинских услуг, отдельные аспекты определения размера возмещения за такой ущерб. Охарактеризованы основания для возникновения ответственности медицинских учреждений или частных врачей за причинение увечья, иного повреждения здоровья или смерти, а также особенности такой ответственности при наличии уголовного производства. Определены общие тенденции в принятии Европейским судом по правам человека решений по делам, касающимся причинения вреда жизни или здоровью лица в сфере медицинских услуг. Охарактеризована роль и особенности судебно-медицинской экспертизы как ключевого основания для установления факта причинения вреда жизни или здоровью пациента.

რეზიუმე

დასახიჩრებით, ჯანმრთელობის სხვა დაზიანებით ან სიკვდილით მიყენებული ზარალის ანაზღაურების ვალდებულებანი: თეორიული და პრაქტიკული ასპექტები (მიმოხილვა)

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ნაშრომის მიზანს წარმოადგენდა დასახიჩრებით, ჯანმრთელობის სხვა დაზიანებით ან სიკვდილით მიყენებული ზარალის ანაზღაურების სამართლებრივი საფუძვლების და თავისებურებების კომპლექსური ანალიზი სამედიცინო მომსახურების გაწევის ან მომსახურებაზე უსაფუძვლო უარის თქმის შედეგად.

ჩატარებულია კომპლექსური კვლევა მიყენებული ზიანის ანაზღაურების რეგულირების საკითხებზე უკრაინის სამოქალაქო კანონმდებლობაში და ამ ტი-

პის დავეების არასასამართლო და სასამართლო საშუალებებით დარეგულირების რეკლამენტაციის ნორმატიული ბაზის შესახებ. კვლევაში გამოყენებულია შედარებით-სამართლებრივი, სისტემურ-კომპლექსური, ანალიზის, ინდუქციის და მოდეირების მეთოდები.

ჩატარებული კვლევის საფუძველზე განსაზღვრულია პაციენტების სიცოცხლისა და ჯანმრთელობის ზიანთან დაკავშირებული დავეების დარეგულირების არასასამართლო და სასამართლო საშუალებები, ზიანის ანაზღაურების მოცულობის განსაზღვრის ცალკეული საკითხები. ნაშრომში განსაზღვრულია ადამიანის უფლებათა ევროპული სასამართლოს გადაწყვეტილებების ზოგადი ტენდენციები ადამიანის უფლებებთან მიმართებით, რომელიც შეეხება ადამიანის სიცოცხლისა და ჯანმრთელობისათვის ზიანის მიყენებას სამედიცინო მომსახურების სფეროში; დახასიათებულია პაციენტის სიცოცხლისა და ჯანმრთელობისათვის ზიანის მიყენების ფაქტის დადგენაში საკვანძო საფუძვლის - სასამართლო-სამედიცინო ექსპერტიზის როლი და თავისებურებანი.

PROTECTION OF HUMAN RIGHTS BY THE CONSTITUTIONAL COURT OF UKRAINE IN THE FIELD OF HEALTH CARE (REVIEW)

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The Constitutional Court of Ukraine is the only body of constitutional jurisdiction in Ukraine [2,3]; its task is to guarantee the supremacy of the Constitution of Ukraine. The Constitutional Court sets out its legal position in reasoning and/or resolution part of the decision and the conclusion. By August 1, 2019, the Constitutional Court of Ukraine made decision in 4 medical cases: the case of K. G. Ustimenko (1997) [4], the case of paid medical services (1998) [6], the case of free medical care (2002) [5], the case of judicial control over the hospitalization of incapacitated persons into psychiatric institution (2016) [7]. At the same time, it is well known that in Ukraine, as well as in other several countries, a thorough reform in the field of protection has been initiated, and it is being continued on now [8]. New laws and by-law documents are being drafted. In order to avoid legal conflicts during their development, on one hand, it is necessary to take into account legal positions of the Constitutional Court of Ukraine. On the other hand, it should be borne in mind that the Constitutional Court of Ukraine may develop and specify its legal position of the Court in its subsequent acts, change legal position of the Court in the its further actions, change its position within the regulatory framework caused by essential change of normative regulation, which the Court was going by, or if there are objective grounds for the necessity of protection of constitutional rights and freedoms improvement, taking into account Ukraine's international obligations, and on condition that such changes are justified in the Court's act.

Thus, the examination of the decisions of the Constitutional Court of Ukraine and the analysis of legal positions contained there in the light of radical reform of the health care sector in Ukraine is relevant, and theoretically and practically useful.

The purpose of this article is to clarify the role and place of the Constitutional Court of Ukraine in the system of judicial protection of human and citizen rights, to determine the prospects of applying legal positions of the Constitutional Court of Ukraine to the development of new legislation of Ukraine in the light of radical health care reform.

Material and methods. The methodological basis of the conducted research is the general methods of scientific cognitivism as well as concerning those used in legal science: methods of analysis and synthesis, formal logic, comparative law etc.

It is commonly known that the Constitutional Court of Ukraine takes into account the prescriptions of current international treaties, the consent of which has been granted by the Verkhovna Rada of Ukraine (Supreme Council of Ukraine), and the practices of interpretation and application of these treaties by international bodies have been recognized by Ukraine [14-16]. These include the Convention for the Protection of Human Rights and Fundamental Freedoms and the case-law of the European Court of Human Rights. The decision of the Constitutional Court of Ukraine was taken in the light of the case-law of the European Court of Human Rights [1].

The Decision of the Constitutional Court of Ukraine in the case of K. G. Ustymenko. The basis for consideration of the case according to the Article 94 of the Law of Ukraine “On the Constitutional Court of Ukraine” was the existence of ambiguous application of the provisions of norms of the Law of Ukraine “On Information” and the Law of Ukraine “On the Prosecutor’s Office” by courts of general jurisdiction, which led to the violation of the constitutional rights and freedoms of citizens of Ukraine. In paragraph 1 of the resolution part of the decision the Constitutional Court of Ukraine stated that part four of Article 23 of the Law of Ukraine “On Information” should be understood as prohibiting not only the collection, but also storage, use and spreading of confidential information about a person without his/her prior consent, except cases determined by law and only in the interests of national security, economic well-being, human rights and freedoms. Confidential information, in particular, includes identity cards (education, marital status, religiosity, health status, date and place of birth, property status and other personal data).

Consent to collect, store, use and spread of information about disabled person is provided by family member or legal representative. During the period of gathering information about him/her, every able person, family member or legal representative of a disabled person has the right to know what information is collected and for what purpose, how, by whom and for what purpose they are used. During the storage and spreading of personal data, those persons have the right to access to such information, to deny its correctness and completeness, etc.

In paragraph 2 of the resolution part of the decision of the Constitutional Court of Ukraine stated that part five of Article 23 of the Law of Ukraine “On Information” should be understood, so that every person has the right to get acquainted with the information about him in state authorities, local self-government bodies, establishments and organizations, if this information is not a state or other legally protected secret. Medical information, i. e. evidence of a person’s health, medical history, purpose of the proposed research and treatment measures, prognosis of possible development of the disease, including the presence of risk to life and health, by its legal regime belongs to confidential, that is to information with limited access. At patient’s request as well as at the request of his or her family members or legal representatives the physician is obliged to provide them with such information in a complete and accessible form.

In special cases, as stipulated in part three of Article 39 of the Fundamentals of the Ukrainian legislation on health care, when complete information may cause harm to the patient’s health, the doctor may restrict it. In this case he is to inform the family member or legal representative of the patient, regarding the patient’s personal interests. The doctor acts in the same way when the patient is being unconscious condition. In case of refusal to provide or intentionally withhold medical information from a patient, his or her family members or legal representative may appeal against the actions or omissions of the doctor directly in the Court or, by their own choice, at the medical institution or health care authority.

In paragraph 3 of the resolution part of the decision the Constitutional Court of Ukraine stated that in Article 48 of the Law of Ukraine “On Information” the norms, formulated in part one of this Article, are decisive and provide for appeal against unlawful acts committed by public authorities, local self-government bodies and their officials, as well as by political parties, other public organizations, mass media, state-owned legal entities and individuals and higher authorities, or to the court, that

is at the choice of the complainant. Part two of Article 48 of the Law of Ukraine “On Information” just establishes the procedure for appealing against unlawful actions of officials in case of appeal to higher level bodies, and part three of this Article emphasizes that the appeal filed to higher level bodies is not an obstacle for further appeal to public or legal entity. The third part in the context of the whole Article 48 of the Law of Ukraine “On Information” cannot be understood as a requirement for mandatory appeal against illegal actions of officials to the higher-level bodies initially and then to the Court. Direct appeal to the Court is everyone’s constitutional right.

This decision of the Constitutional Court of Ukraine is the first example of the protection of the constitutional rights of an individual by exercising constitutional powers as for formally interpret of the Constitution and the Laws of Ukraine, since at that time there was no Constitution of Ukraine and the Law of Ukraine “On the Constitutional Court of Ukraine” of 16 October 1996, where the right of individuals to constitutional complaint had not been enshrined. These persons could apply to the Constitutional Court of Ukraine only indirectly through the following subjects of the right to a constitutional submission: the President of Ukraine, not less than 45 people’s deputies of Ukraine, the Supreme Court of Ukraine, the Ombudsman of the Verkhovna Rada of Ukraine, the Verkhovna Rada of the Autonomous Republic of Crimea.

In accordance with the correction put to the Constitution of Ukraine, everyone is guaranteed the right to file a constitutional complaint to the Constitutional Court of Ukraine on the grounds established by the Constitution and in accordance to legally established procedure (Article 55). The Constitutional Court of Ukraine resolves the issue of conformity to the Constitution of Ukraine (constitutionality) with the law of Ukraine on the constitutional complaint of a person who believes that the law of Ukraine applied in the final Court decision in its case is contrary to the Constitution of Ukraine. A constitutional complaint may be lodged if all other domestic methods have been exhausted (Article 151¹).

The decision of the Constitutional Court of Ukraine in the case of paid medical services. On September 17, 1996, the Cabinet of Ministers of Ukraine adopted Resolution No. 1138 “On Approving the List of Paid Services Provided at State Health Care Institutions and Higher Medical Educational Establishments”, which approved the mentioned list and allowed medical and sanitary-preventive institutions to receive from patient’s payment for other medical services as their voluntary compensation [10]. The subject of the right to constitutional submission, considering that the List of paid services actually includes medical, preventive and other types of medical care, which in accordance with part 3, Article 49 of the Constitution of Ukraine that should be provided free of charge in this kind of establishments, appealed to the Constitutional Court and requested The Constitutional Court of Ukraine to admit the Cabinet of Ministers of Ukraine of September 17, 1996, with the following amendments as unconstitutional.

The Constitutional Court of Ukraine formulated the following legal positions: 1) the way out of the critical situation with the budget financing of health care, not in introducing practically unlimited list of paid medical services, but, in changing according to the above mentioned provisions of the Constitution of Ukraine, conceptual approaches to the solution of problems related to ensuring the constitutional right for medical care, developing, approving and implementing appropriate nationwide programs that would clearly identify the state-guaranteed (in-

cluding public funding) the amount of free medical care for all citizens in state and municipal health care institutions, the introduction of health insurance, etc. (paragraph 13, clause 2 of the motivation part); 2) the charitable activity must be carried out in the appropriate legal forms and in the order provided, in particular, by the Law of Ukraine “On Charity and Charitable Organizations” of September 16, 1997 (sentence 2, paragraph 2, clause 3 of the motivation part).

The decision of the Constitutional Court of Ukraine in the case of free medical care. The subject of the right For constitutional petition – People’s Deputies of Ukraine – appealed to the Constitutional Court of Ukraine with a request for the official interpretation of the term “free medical care” and the provisions of part 3, Article 49 of the Constitution of Ukraine that “in state and communal health care establishments medical aid is free of charge” as well as raising the following question: should medical aid be paid only at the expense of the state budget and employer-based health insurance, excluding other solidarity forms of involvement of public funds; should this constitutional norm be understood as “that is why health care in public and communal health care facilities can be provided not only through state budget funding and employers’ contributions, but also through the mobilization of the population costs through other solidarity forms such as hospitals cash desks, credit unions, etc.” [5].

Investigating the case file, the Constitutional Court of Ukraine formulated the following positions [5]. Thus, the term “free of charge” (in the text of Article 49 of the Constitution of Ukraine it is “free of charge”), the official interpretation of which is requested by the People’s Deputies of Ukraine, has no independent meaning. Its meaning is determined either by the context or the logical interconnection of the words in which it is used. In the phrase “medical aid is provided free of charge”, the last word in the context of the whole Article 49 of the Constitution of Ukraine means that an individual receiving such an assistance in state and communal health care institutions should not reimburse its cost in any kind of payments or in any form whatever the time of provided care. This lies completely in line with the meaning of the word “free”: that is not paid, what is not paid does not need to be paid; what is not charged; that which received no remuneration; that has synonyms “unpaid”, “unselfish”; something that has an antonym to pay in any form. An example of the latter opposition is given in the part 2, Article 47 of the Constitution of Ukraine: “Citizens, who are in need of social protection, are provided housing free of charge by the state and local self-government bodies, or at a fee available to them in accordance with the law.”

Also, the Constitutional Court of Ukraine formulated the position according to which part one of Article 49 of the Constitution of Ukraine enshrines the right of everyone to health insurance, i. e. not compulsory but voluntary health insurance of citizens. With regard to state health insurance, its introduction will not contradict the constitutional prescription “in state and communal health care establishments medical aid is provided free of charge” only if the payers of compulsory insurance payments (contributions) are organizations, institutions, enterprises, others business entities engaged into business activities, government funds, etc. The collection of such kinds of payments (contributions) from citizens in the public health insurance system goes out of conformity with the constitutional provision, as it is to be the form of payment for their assistance in state and communal health care institutions.

Mentioned-above does not prohibit the provision and the possibility of providing citizens with medical services that go be-

yond the scope of medical care (according to the terminology of the World Health Organization – “secondary medical services”, “paramedical services”), for a fee in these institutions. The list of such paid services cannot be invaded within the limits of free medical care and in accordance with the requirements of paragraph 6 of part one of Article 92 of the Constitution of Ukraine, it must be established by law.

The provision “in state and communal health care facilities it is provided free of charge” applies to all such establishments that are publicly owned (regardless of departmental ownership) or communal property and financed from budgets of any level.

Considering, in the light of such provisions, the issue of the possibility of joint participation of the population in additional financing of the healthcare sector, declared by the subject of the right to a constitutional submission, the Constitutional Court of Ukraine notes that free health care in state and communal health care institutions does not exclude such opportunities through the financing of this industry through the development of extra-budgetary mechanisms to raise additional funds, including through the establishment of hospital mutual help cash desks (unions, funds), which activities should be regulated by law. The sources of such additional revenues for health care financing in general may be statutory official direct payments of the population for medical services of secondary importance, and departmental appropriations for the maintenance of medical institutions, and benefits from charity actions and events, and funds from international humanitarian programs. assistance and donations from public, religious charities and philanthropists, employers’ contributions to the compulsory state health insurance system, finances of voluntary health insurance companies, territorial community savings programs resources, and government medical loans, etc. (paragraphs two, three, eighth, ninth, eleventh of clause 4, first, second paragraph 5 of the motivation part of the Decision).

The Constitutional Court of Ukraine also formulated the position according to which the provisions of Part 3, Art. 49 of the Constitution of Ukraine “in state and communal health care facilities medical aid is provided free of charge” should be understood that in state and communal health care facilities medical care is provided to all citizens regardless of its content and without their previous, current or subsequent calculation for providing such an assistance. The concept of health care, conditions for introduction of health insurance, including state one, the formation and use of voluntary medical funds, as well as the procedure for the provision of medical services that go beyond medical care, on paid basis in state and communal health care institutions and the list of such services must be defined by the law.

Health care reform has begun and is currently underway in Ukraine [8,9,17]. In August 2014, the Ministry of Healthcare of Ukraine initiated the working out of the National Strategy for Healthcare Reform in Ukraine for the period of 2015-2020. It became the part of the National Action Plan on the Total Reform of the State Health Care System, which was proclaimed by the Decree of the President of Ukraine in January 12, 2015 “On the Sustainable Development Strategy” Ukraine 2020” and by the Government of Ukraine. The main goals of thorough healthcare reform in Ukraine are the following: changing the concept of financing; the formation of hospital districts; transformation of health care establishments from budgetary institutions into communal non-profitable ones [11,12,18,19].

On October 19, 2017, the Law of Ukraine “On State Financial Guarantees of Public Health Services” was adopted [20]. The most debatable issue is the question of a new order for financ-

ing medical services. The concept of such financing is set out in detail by the Cabinet of Ministers of Ukraine "On Approval of the Concept of Health Care Financing Reform". Three payment systems have been introduced [8]. According to its contents, the list of such services is to be significantly expanded, and the list of medical services that requires partial payment will appear. Such measures can be considered as narrowing rights guaranteed by the Constitution of Ukraine to free medical care in state and municipal health care institutions. To solve the problem of contradiction in the Art. 49 of the Constitution of Ukraine and the involvement into the field of health care for citizens the compulsory social health insurance as a special kind of budgetary (consolidated) funds. In order to comply with the requirements of the Constitution of Ukraine and expand the sources of funding for health care, employers must pay mandatory health insurance contributions. This indicates the need for the Verkhovna Rada of Ukraine to adopt the Law of Ukraine on Compulsory Social Health Insurance.

The decision of the Constitutional Court of Ukraine in the case of judicial control over the hospitalization of incapacitated persons into psychiatric institute. The subject of the right for constitutional petition, that is the Commissioner of the Verkhovna Rada of Ukraine for Human Rights, appealed to the Constitutional Court of Ukraine to declare the provision of the third sentence of part one of Article 13 of the Law of Ukraine "On Psychiatric Assistance" dated February 22, 2000 (hereinafter - the Law) with the corrections, which stipulate person to be recognized as incapacitated and hospitalized at the request of psychiatric institution or with the consent of his/her guardian.

The Constitutional Court of Ukraine stated that restrictions on the exercise of constitutional rights and freedoms cannot be arbitrary and unjust, they must be established exclusively by the Constitution and Laws of Ukraine, they are to pursue a legitimate aim, be conditioned by the public need to achieve this goal, proportionate and justified, if Constitutional law or freedom of the legislator is obliged to introduce such legal regulation that is to allow to achieve optimally legitimate goal with minimum intrusion into the exercise of this right or freedom and not violating the substantive content of this very right (paragraph 2.1 of the resolution part of the Decision).

In the opinion of the Constitutional Court of Ukraine, the recognition of a person as incapable cannot deprive him or her of other constitutional rights and freedoms or restrict them in a way that negates their essence (paragraph 2.2 of the resolution part of the Decision). Although incapacitated persons are incapable of personally exercising individual constitutional rights and freedoms, including the right to liberty and security of person, they cannot be completely deprived of these rights and freedoms, so the state is obliged to create effective legislative mechanisms and guarantees for their maximum implementation. The right to liberty means that a person is free in his or her activities from outside interference, except for the restrictions established by the Constitution and Laws of Ukraine (paragraph 2.3 of the motivating part of the Decision).

The legal position of the Constitutional Court of Ukraine is the need to exercise judicial control over the interference with the right to liberty and personal inviolability of a mentally ill person during his or her hospitalization into a psychiatric institution without his or her consent (paragraph 2.3 of the motivation part of the Decision). Hospitalization of an incapacitated person into a psychiatric institution on the basis of Art. 13 of the Law is a restriction on his/her right to freedom and personal integrity, enshrined in Art. 29 of the Constitution of Ukraine, and there-

fore it must be provided in accordance with the criteria set out in this decision.

The procedure established by the Law for the hospitalization of an incapacitated person into psychiatric institution at the request or with the consent of her/her guardian by decision of psychiatrist does not presume judicial control over this kind of hospitalization, since the legislator did not consider it as voluntary one, although hospitalization of an incapacitated person takes place without his or her informed consent (p. 2.5 of the motivational part of the Decision). The Constitutional Court of Ukraine considers that such a hospitalization by its nature and consequences is disproportionate restriction of the constitutional right of an incapacitated person to freedom and personal integrity, and therefore it must be carried out in compliance with the constitutional guarantees of the protection of human and citizen's rights and freedoms, taking into account the above-mentioned international standards, legal positions of the Constitutional Court of Ukraine, and exclusively by the Court decision in accordance with the requirements of Art. 55 of the Basic Law of Ukraine (paragraph 2.5 of the motivation part of the Decision).

Judicial control over the hospitalization of an incapacitated person into psychiatric institution in accordance with the procedure provided by Art. 13 of the Law, is necessarily guarantee protection of individual's rights and freedoms enshrined, in particular, in Art. 29, 55 of the Basic Law of Ukraine. The court after independent and impartial consideration of the issue of hospitalization of an incapacitated person into psychiatric institution must decide on the lawfulness of such person's constitutional right to liberty and personal inviolability (paragraph 2.5 of the motivating part of the Decision).

Conclusions.

1. The basic principles on the protection of human rights in the field of health care formulate general basis for constitutions of democratic countries. The Constitutional Court of Ukraine plays a key role in guaranteeing human rights in the field of health care. Legal positions of the Constitutional Court, based on these principles, and that are being used in one country, taking into account the particularities of national law, can be an inspiration to the courts of other countries.

It is substantiated that taking into account the legal positions of the Constitutional Court of Ukraine on free medical care, paid medical services, collecting, storing, using and spreading information, in particular about the mental state of a person, his or her compulsory examination and treatment, judicial control over the hospitalization of incapacitated persons into psychiatric institution is necessary to avoid judicial conflicts when working out over health care legislative bills in the context of radical reform, which is going on in Ukraine.

2. Attention is drawn to the fact that until recent times the forms of addressing to the Constitutional Court of Ukraine were constitutional petitions and constitutional appeals. Some amendments have been introduced into the Constitution of Ukraine not long ago, in particular the concept of constitutional complaint has been introduced. The institution of Constitutional Complaints allows to exercise the right of individual access to constitutional justice. This will help to ensure the supremacy of law, protection of human rights and freedoms in relations with the state.

3. The legal positions of the Constitutional Court of Ukraine in the following medical cases were revealed: the case of K. G. Ustyenko (1997), the case of paid medical services (1998), the case of free medical care (2002), the case of judicial control over the hospitalization of incapacitated persons into psychiatric institution (2016).

4. It is established that the Health Care Financing Reform Concept introduces three payment systems: full state compensation; partial state compensation (co-payment is made by citizens independently); full payment for some types of medical services by the patient on his/her own and as a result the list of paid medical services are to be substantially expanded, the list of medical services that are to require partial payment will appear. It is proved that these measures can be regarded as nothing more than narrowing of the right guaranteed by Article 49 of the Constitution of Ukraine to free medical care in state and communal health care establishments. It is argued that to resolve the contradiction of Art. 49 of the Constitution of Ukraine and involvement in the field of health care of citizens may either amend the Constitution of Ukraine, according to which state and communal health care institutions would have the right to provide paid health services, or introduce compulsory social health insurance as a special kind of budgetary (consolidated) costs.

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SUMMARY

PROTECTION OF HUMAN RIGHTS BY THE CONSTITUTIONAL COURT OF UKRAINE IN THE FIELD OF HEALTH CARE (REVIEW)

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The article explores the issue of human rights protection in the field of health care by the Constitutional Court of Ukraine. The decisions of the Constitutional Court of Ukraine in the case of K. G. Ustimenko (1997), the case of paid medical services (1998), the case of free medical care (2002), the case of judicial control over the hospitalization of incapacitated persons into psychiatric institute are analyzed (2016), as well as the new legislation of Ukraine in the light of radical reform in the healthcare sector. Attention is drawn to the principle of friendly attitude to international law, constitutional complaint, legal positions of the Constitutional Court of Ukraine in the above-mentioned cases and the prospect of their application into the development of new legislation of Ukraine in the context of radical reform of the health care system of Ukraine.

It is emphasized that the legal positions of the Constitutional Court of Ukraine, with regard of peculiarities of the national legislature, can serve as a source of inspiration for the courts of other countries.

The purpose of the article is to specify the role and place of the Constitutional Court of Ukraine in the system of judicial protection of human and citizen rights, to determine the prospects of applying the legal positions of the Constitutional Court of Ukraine within the development of new legislation of Ukraine in the light of radical reform of the health care system.

The object of the study is the social relations that arise during protection of human rights in the field of health care by the Constitutional Court of Ukraine. The methodological basis of the research are general and special methods of scientific knowledge (formal-logical method, comparative-legal, structural-logical).

As a result of the conducted research, the role and place of the Constitutional Court of Ukraine in the system of judicial protection of human and citizen rights, the role of the decisions of the Constitutional Court of Ukraine in the case of K. G. Ustimenko, the case of paid medical services, the case of free medical care, the case of judicial control over hospitalization of incapacitated persons into psychiatric institution in the formation and development of domestic constitutional proceedings are defined. It is emphasized that the introduction of the constitutional complaint concept (institution) contributed to the improvement of the national mechanism of human rights protection in the field of health care. Conflicts of constitutional regulation of the human right to free medical care have been identified, and proposals have been worked out regarding possible ways and methods to eliminate them.

Keywords: protection of human rights in the field of health-care, Constitutional Court of Ukraine, constitutional complaint, legal position, free medical care, medical service, hospitalization of incapacitated persons, psychiatric care.

РЕЗЮМЕ

ЗАЩИТА ПРАВ И СВОБОД ЧЕЛОВЕКА В СФЕРЕ ЗДРАВООХРАНЕНИЯ КОНСТИТУЦИОННЫМ СУДОМ УКРАИНЫ (ОБЗОР)

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В статье исследуется вопрос защиты прав человека в сфере здравоохранения Конституционным Судом Украины. Анализируются решения Конституционного Суда Украины в деле К.Г. Устименко (1997 г.), по делам о платных медицинских услугах (1998 г.), бесплатной медицинской помощи (2002 г.), судебном контроле за госпитализацией недееспособных лиц в психиатрическое учреждение (2016 г.), а также принятое новое законодательство Украины в свете коренной реформы сферы здравоохранения. Акцентируется внимание на принципе дружественного отношения к международному праву и на вопросах конституционной жалобы, юридических позициях Конституционного Суда Украины в вышеуказанных делах и перспективе их применения при разработке нового законодательства Украины в контексте коренной реформы системы здравоохранения.

Подчеркивается, что юридические позиции Конституционного Суда Украины с учетом особенностей национального законодательства могут служить источником инспирации для судов других стран.

Целью проведенного исследования является уточнить роль и место Конституционного Суда Украины в системе судебной защиты прав человека и гражданина, определить перспективы применения правовых позиций Конституционного Суда Украины в разработке нового законодательства в свете коренной реформы системы здравоохранения. Объектом исследования являются общественные отношения, возникающие при защите Конституционным Судом Украины прав че-

ловека в сфере здравоохранения. Методологической основой проведенного исследования являются общие и специальные методы научного познания (формально-логический метод, сравнительно-правовой, структурно-логический).

В результате проведенного исследования определены роль и место Конституционного Суда Украины в системе судебной защиты прав человека и гражданина, роль решений Конституционного Суда Украины в деле К.Г. Устименко, деле о платных медицинских услугах, в деле о бесплатной медицинской помощи и судебном контроле за госпитализацией недееспособных лиц в психиатрическое учреждение, в становлении и развитии отечественного конституционного судопроизводства. Акцентировано, что введение института конституционной жалобы содействует усовершенствованию национального механизма защиты прав человека в сфере здравоохранения. Выявлены коллизии конституционного регулирования права на бесплатную медицинскую помощь, разработаны предложения относительно возможных способов и путей их устранения.

რეზიუმე

ჯანმრთელობის დაცვის სფეროში ადამიანის უფლებებისა და თავისუფლების დაცვა უკრაინის კონსტიტუციური სასამართლოს მიერ (მიმოხილვა)

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შესწავლილია ადამიანის უფლებათა დაცვის საკითხი ჯანმრთელობის სფეროში უკრაინის კონსტიტუციური სასამართლოს მიერ. გაანალიზებულია უკრაინის კონსტიტუციური სასამართლოს გადაწყვეტილებები კ.უსტიმენკოს საქმეზე (1997 წ.), ფასიანი სამედიცინო მომსახურების (1998 წ.), უფასო სამედიცინო მომსახურების (2002 წ.), ქმედუნარო პირების ფსიქიატრიულ დაწესებულებაში ჰოსპიტალიზაციის სასამართლო კონტროლის (2016 წ.) საქმეებზე და უკრაინაში მიღებული ახალი კანონმდებლობა ჯანდაცვის სფეროს ძირეული რეფორმის ჭრილში. ეურადღება აქცენტირებულია დამოკიდებულებაზე საერთაშორისო სამართალთან და კონსტიტუციური დაცვის საკითხებზე უკრაინის კონსტიტუციური სასამართლოს იურიდიულ პოზიციებზე ამ საქმეებთან დაკავშირებით და მათი გამოყენების პერსპექტივაზე უკრაინაში ჯანდაცვის სფეროს ახალი კანონმდებლობის კონტექსტში. ხაზგასმულია, რომ უკრაინის კონსტიტუციური სასამართლოს იურიდიული პოზიციები ეროვნული კანონმდებლობის თავისებურებების გათვალისწინებით შესაძლოა ინსპირაციის წყარო გახდეს სხვა სახელმწიფოების სასამართლოებისათვის.

ჩატარებული კვლევის მეთოდოლოგიურ საფუძველს წარმოადგენდა სამეცნიერო კვლევის საერთო და სპეციფიკური მეთოდები (ფორმალურ-ლოგიკური, შედარებით-სამართლებრივი, სტრუქტურულ-ლოგიკური მეთოდები).

კვლევის შედეგად განსაზღვრულია უკრაინის კონსტიტუციური სასამართლოს როლი და ადგილი ადამიანისა და მოქალაქის უფლებათა სასამართლო დაცვაში, უკრაინის კონსტიტუციური სასამართლოს როლი

კუსტიმენკოს საქმეში, ფასიანი სამედიცინო მომსახურების, უფასო სამედიცინო მომსახურების ქმედუნარო პირების ფსიქიატრიულ დაწესებულებაში ჰონსპიტალიზაციაზე სასამართლო კონტროლის საქმეებში, ეროვნული კონსტიტუციური სასამართლო საქმისწარ-

მოების შექმნასა და განვითარებაში. გამოვლენილია კოლიზიები თითოეული ადამიანის მიერ უფასო სამედიცინო დახმარების მიღების უფლების რეგულაციაში, შემუშავებულია წინადადებები მათი აღმოფხვრის საშუალებებსა და გზებთან დაკავშირებით.

РЕЗУЛЬТАТЫ ПРЕДВАРИТЕЛЬНОГО ИССЛЕДОВАНИЯ РАСТЕНИЙ ФЛОРЫ ГРУЗИИ НА СОДЕРЖАНИЕ ФЛАВОНОИДОВ И ТРИТЕРПЕНОИДОВ

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Фитохимические исследования, проводимые в период 1960-2017 гг. в отделе фитохимии (ныне департамент фитохимических исследований, направление „Фенольные соединения“), Института фармакохимии им. И.Г. Кутателадзе, позволили выявить целый ряд растений с богатым содержанием биологически активных соединений. Особый интерес представляют виды семейств: *Fabaceae*, *Rosaceae*, *Umbelliferae*. Среди них заслуживают внимания виды рода *Astragalus*, *Trifolium*, представители которых являются сырьем официальных лекарственных препаратов и БАД [3-5]. Созданные на их основе препараты проявляют высокую антиоксидантную, гипогликемическую, гипоазотемическую, гипохолестеринемическую активность [3].

Суммарные субстанции из некоторых видов рода *Astragalus*, *Eupatorium* проявляли лейкопозную активность [3], *Ononis* - диуретическую и гастропротекторную активность. Поиск индивидуальных веществ, обладающих этим действием, представляет интерес для исследователей-ученых не только Грузии, но и зарубежья [2]. Одни и те же растения флоры Грузии в зависимости от эколого-климатических условий страны обладают различным друг от друга химическим составом как в качественном, так и количественном плане, что оказывает влияние на степень их биологической активности.

Целью исследования явилось проведение предварительного химического анализа растений, собранных в результате целевых фармакоботанических экспедиций института, выявление объектов с высоким содержанием флавоноидов и тритерпеноидов и их дальнейшее углубленное химическое и фармакологическое исследования.

Материал и методы. Объектом исследования являлись воздушно-сухие вегетативные органы растений (листья, семена, цветы, стебли, корни), собранных за период 2016-2017 гг. Измельченное сырье экстрагировали 80% этанолом при нагревании на водяной бане с обратным холодильником в соотношении 1:10. Органический растворитель из экстракта отгоняли на вакуум-ротационном испарителе; водный остаток очищали хлороформом до полного удаления липофильных веществ; хлороформенные извлечения

стужали, остаток растворяли в метаноле и анализировали аналогично водному остатку. Качественные реакции на флавоноиды проводили по Вруант [2]. Интенсивность реакции обозначали знаком „+“ по пятибалльной системе. Водные и хлороформенные фазы наносили на пластинки силуфол УФ 254 и проявляли в системах н-бутанол-уксусная кислота-вода в разведении 4:1:2 и хлороформ-метанол 10:1 [2]. Пластинки сушили при комнатной температуре и детектировали 10% метанольным раствором гидроокиси калия (флавоноиды) и 25% метанольным раствором фосфорновольфрамовой кислоты (терпеноиды).

Geranium ibericum и *Polygonum corneum* ввиду богатого содержания флавоноидов подвергали более глубокому анализу. Выделенные вещества идентифицировали посредством изучения физико-химических свойств, продуктами химического превращения, данными инфракрасной (ИК), ультрафиолетовой (УФ)-спектроскопией, сравнивая с рабочим стандартом.

Результаты и их обсуждение. Проанализирован 341 образец 117 (106 разных) растений, относящихся к 93 родам и 36 семействам (таблица) [8].

Среди исследованных растений виды *Astrantia maxima*, *Laser trilobum* (сем. *Apiaceae*), *Otanthus maritimus* (сем. *Asteraceae*), *Capparis spinosa* (сем. *Capparaceae*), *Laburnum anagyroides* (сем. *Fabaceae*), *Lavatera thuringiaca* (сем. *Malvaceae*), *Atrapaxis caucasica*, *Polygonum carneum* (сем. *Polygonaceae*), *Geranium ibericum* (сем. *Geraniaceae*), *Euphrasia stricta* (сем. *Scrophulariaceae*) ранее не анализировались. В исследуемых объектах богатым содержанием флавоноидов характеризуются растения сем. *Asteraceae*, *Apiaceae*, *Caprifoliaceae*, *Cruciferae*, *Gentianaceae*, *Fabaceae*, *Labiatae*, *Liliaceae*, *Rubiaceae*, *Rosaceae*, *Malvaceae*, *Umbelliferae* и *Vallerianaceae* (таблица).

По содержанию флавоноидов особого внимания заслуживают *Geranium ibericum*, *Polygonum carneum*, *Consolida divaricata*, *Rhus coriaria*, в которых обнаружены производные кверцетина, кемпферола и мирицетина. Другие виды рода *Geranium* - *G. pusillum* и *G. Robertianum* ранее изучены на содержание флавоноидов и конденсированных танинов [8,9].

Таблица. Предварительное исследование растений флоры Грузии на содержание флавоноидов и тритерпеноидов

№	Семейство, Род, Вид	Место и время сбора	Исследо- мые части	Флавоноиды			Тритерпеноиды (циклоартаны), реакция с 25% фосфорновольфрамовой кис-той [2]
				Реакция по Bryant [2]	Коли- чес- тво пятен	Rf интенсивных пятен	
1	2	3	4	5	6	7	8
1.	Amaranthaceae Amaranthus hybridus L.	Горийский р-н, с. Хидистави, 2016	Листья Цветки Стебли Корни	+ + + -	- - - -	- - - -	- - + -
2	Anacardiaceae Rhus coriaria L.	Тбилиси, левый берег р. Вере, 2017	Стебли Плоды	++++ +++++	9 6	0,53; 0.59 0.54	+++ +++
3	Asteraceae Bidens cernua L.	Побережье черного мо- ря, окр.Маг- нетити, 2016	Листья Цветки Стебли Корни	++++ ++ ++ +?	5 6 4 4	0.55;0,63;0.66; 0.75;0.86 0.51;0.63;0.83 - -	- - - -
4	Gnaphalium sp.	Бакуриани, окр. Дидве- ли, 2017	Надз.часть	++++	11	0,66; 0,82	++
5	Grossheimia macrocephala (Muss.-Puschk. ex Willd) Sosn. Takht.	Горийский р-н, с. Хидистави 2016	Листья Цветки Стебли Корни	+++++ +++++ ++ -	6 7 3 4	0.60;0.69;0.79 0,60;0.75;0.85- - -	+ + +++ +++
6	Inula germanica L.	Тбилиси, окр. Глдани, 2016	Листья Цветки Стебли Корни	++++ +++++ ++ -	5 8 6 -	0.46;0.54;0.61;0.80; 0.87 0.58; 0.79 0.46;0.70;0.87 -	+ ++ + +
7	Lapsana grandiflora Bieb.	Окр. оз. Паравани, 2017	Листья Цветки Стебли Корни	+++++ +++++ +++++ -	7 8 5 5	0,46;0,67;0.84 0.46;0,67;0,87 - -	- - +++ следы
8	L. communis L.	Хобский р-н, Квалони, 2017	Листья Цветки Стебли	- - -	5 4 4	0.62; 0.80 - 0.80	следы следы +
9	Otanthus maritimus (L.) Hoffmigg & Link	Аджара, Чолоки, 2017	Листья, стебли Цветки, семена Корни с корневи шей	+++ - - -	6 3 6	0,26 - 0.24;0.72; 0.96	+ - ++
10	Petasites albus (L.) Gaertn.	Бакуриани, окр. Дидве- ли, 2016	Листья Стебли Корни	+++++ + -	4 3 4	0,80 - 0.84	+ + +++
11	Senecio sp.	Бакуриани, окр. Дидве- ли, 2016	Листья Цветки Стебли Корни	++++ ++++ + -	4 4 3 2	- - - -	+ ++ - -
12	Serratula radiata (Waldst. &Kit.) Bieb.	Окр. Давид- Гареджи, 2016	Листья Цветки Стебли	++++ +++++ +++	4 4 5	- 0.56;0.84 0.09;0.30;0.45	+ ++ -

13	<i>Sigesbeckia orientalis</i> L.	Горийский р-н.с.Хидистав, 2016	Листья Цветки Стебли Корни	++ ++ - +?	3 3 5 4	- - - -	- - + -
14	<i>Solidago rupes-tris</i> Raf.	Горийский р-н.с. Хидистави, 2016	Листья Цветки Стебли	+++ ++++ +	3 4 2	0.65;0.75;0.82 0.70;0.75;0.82 0.61;0.68	- - ++
15	<i>Tussilago far-fara</i> L.	Горийский р-н, пр. Бе-рег р. Тана, 2016	Листья	++++	5	0,69; 0,75	+
16	Balsaminaceae <i>Impatiens noli-tangere</i> L.	Тианетское шоссе, левая сторо-на, 2016	Листья Стебли Корни	+ +? -	2 2 -	- - -	- ++ +++
17	Betulaceae <i>Betula litwino-wii</i> Doluch.	Бакуриани, Дидвели, 2016	Листья	+++	6	0.72	+++
18	Boraginaceae <i>Echium rubrum</i> Jacq.	Тетрицкаро, окр. Храм-геси-2, 2016	Листья Цветки Стебли Корни	++++ ++ +++ -	9 9 9 9	- 0,89 0,56;0,64;0,76; 0,87 -	- + ++ ++
19	Campanula-ceae <i>Asyneuma cam-panuloides</i> (Bieb.ex Sims) Bornm.	Окр. оз. Паравани, 2017	Листья Цветки Стебли	+ +++ -	7 9 4	- 0,66; 0,75 -	- ++ +++
20	Capparaceae <i>Capparis spi-nosa</i> L	Окр. оз. Джандари, 2017	Листья Цветки Стебли	++++ ++++ ++++	4 4 2	0,45 0,49;0,61;0,70 -	++ ++ ++
21	Caprifoliaceae <i>Sambucus nigra</i> L.	Земо Нич-биси, 2016	Листья Стебли	++++ +	6 2	0,62 -	++ +
22	<i>Viburnum opu-lus</i> L.	Тбилиси, Вашлид-жвари, 2017	Плодовые побеги	++	9	0,59; 0,78	++
23	Caryophylla-ceae <i>Silene wallich-ana</i> Klotzsch.	Окр. оз. Паравани, 2017	Листья Плоды , семена Стебли	++ +++ ++	7 8 6	- - -	- - -
24	Convolvulaceae <i>Convolvulus cantabrica</i> L.	Окр. ущ. Цавкиси, 2016	Листья Стебли Корни	+++ ++++ -	4 5 -	0.65;0,80 0.42;0.56;0,65;0,88 -	- - +++
25	Crucifereae <i>Hesperis matronalis</i> L.	Бакуриани, 2016	Листья Цветки Стебли	++++ ++++ +	8 6 1	0,20;0,28;0,56; 0,66 ;0,72 0,70;0,79;0,87 -	- - -
26	<i>Rapistrum rugosum</i> (L.) All.	Горийский р-н, окр. с. Квахвтели, 2016	Листья Плоды Стебли Корни	++++ ++ ++++ -	5 - 6 -	0,63;0,72;0,80 - 0,60;0,68;0,77 -	++ + + +

27	Cucurbitaceae <i>Bryonia alba</i> L.	Побережье Черного моря, окр. Магнетити, 2016	Листья Плоды Цветки Стебли	+++ - +++ ++	4 - 3 5	0,60 - 0,60 -	- - - +
28	Dioscoreaceae <i>Tamus communis</i> L.	Тбилиси, окр. Цодорети, 2016	Листья Стебли	+++++ +	8 3	0,68;0,82;0,89 -	- +++
29	Equisetaceae <i>Equisetum major</i> L.	Хребет Гомбори, 2017	Надз. часть	+++++	12	0,40; 0,75	+
30	Fabaceae <i>Acacia dealbata</i> Link.	Окр. Кобулеги, 2017	Листья Цветки Стебли Семена	++++ +++++ +++++ ++++	4 3 5 4	- 0,09 - 0,53	- - + +
31	<i>Astragalus galegiformis</i> L.	Тетрицкаройский р-н, окр. с.Орбети, 2017	Листья Цветки Стебли Корни	+++++ +++ - -	9 9 2 4	0,00;0,55;0,67;0,92 0,00;0,02;0,06; 0,12;0,22;0,85 - 0,97	- - ++ -
32	<i>A. maximus</i> Willd.	Цивгомбори, с.Аскилаури, 2016	Листья Плоды Цветки Стебли	+ - +++++ -	3 - 4 -	- - 0,57; 0,70 -	- - ++ +
33	<i>A. adzharicus</i> M.Pop.	Аджара, окр. Шушани, 2016	Листья Стебли (зеленые) Стебли (коричн.) Корни	+++ +++ +++ -	5 7 5 -	0,09; 0,41 0,09; 0,27;0,39 0,53 0,47 -	- - + +
34	<i>A. sommieri</i> Freyn.	Аджара, Хихаты, 2016	Цветки Усики Стебли Корни	+ +++++ - -	3 5 2 -	0,66 0,33; 0,55;0,64 - -	++ - ++ +
35	<i>A. glycyphyllos</i> L.	По дороге Коджори, 2016	Листья Плоды Стебли Корни	++++ +++++ - -	3 5 1 -	- 0,62;0,71;0,76 - -	+ + + +
36	<i>Cercis siliquastrum</i> L.	Тбилиси, Вашлид-жвари, 2017	Листья Стебли	+++ -	9 1	0,62; 0,79 -	- +++
37	<i>Cytisus caucasicus</i> Grossh.	Зедазени, 2017	Лист, ст. Плоды	+++++ ++	14 15	0,43;0,48;0,53 0,78	- ++
38	<i>C. caucasicus</i> Grossh.	Норио, 2017	Стебли Цветки	++++ +++++	3 11	0,38; 0,90 0,21;0,32;0,42; 0,51; 0,60	- +
39	<i>Galega orientalis</i> Lam.	Тетрицкаройский р-н, окр. Храмгеси-2, 2017	Листья Стебли	+? -	6 5	- -	+ ++
40	<i>Goebelia alopecuroides</i> (L.) Bunge.	Горийский р-н, до с. Хидистави, 2016	Листья Цветки Стебли	- +? +?	1 1 3	- - -	- - -
41	<i>Laburnum anagiroides</i> L.	Тбилиси, Вашлид-жвари, 2017	Листья Стебли	+++++ +++	12 2	0,52;0,61;0,69; 0,80 0,94	- -

42	<i>Lathyrus roseus</i> Stev.	Тбилиси, окр. Цкнети 2017	Листья Плоды Стебли	+++ +++ -	4 6 3	- 0,18 -	+ ++ ++
43	<i>Melilotus officinalis</i> (L.)Pall.	окр. Манглиси 2016	Листья Цветки Стебли	+++ + +++	6 3 5	0,50 - 0,10; 0,50	- ++ +
44	<i>M. officinalis</i> (L.)Pall.	Тбилиси, окр.Цкнети, 2017	Цветки Лист, ст.	+++ ++	9 6	0,39;0,52;0,98 0,21;0,35;0,44	+ +++
45	<i>Robinia pseudoacacia</i> L.	Тбилиси, Вашлиджвари, 2017	Цветки белые	++++	7	0,62	+++
46	<i>R.pseudoacacia</i> L.	Горийский р-н.с.Хидистави, 2017	Цветки розовые	++++	8	0,55;0,61; 0,67 0,73;0,77;0,82	+++
47	<i>R. pseudoacacia</i> L.	Горийский р-н. с. Хидистави, 2016	Лист,ст.	+++	9	0,06;0,37; 0,530,63	-
48	<i>Sophora</i> sp.	окр. Тбилиси, 2017	Цветки	++++	8	0,04;0,23;0,38; 0,53	++
49	<i>S. japonica</i> L.	окр. Тбилиси, 2016	Листья, цветки Плоды Стебли	++ +++++ -	4 8 3	0,37; 0,69 0,57; 0,69;0,74 0,79; 0 85 0,37; 0,68;0,80	+ - ++
50	<i>Trifolium pratense</i> L.	Бакуриани окр. Дидвели, 2017	Лист, ст. Цветки	++++ ++++	12 12	0,55; 0,63 0,58; 0,69	- -
51	<i>Vicia sativa</i> L.	Горийский р-н.с .Хидистави 2016	Лист,цветки Стебли	+++++ +	2 4	0,91 -	+ ++
52	Geraniaceae <i>Geranium iberica</i> Cav.	окр. оз. Паравани, 2017	Листья Цветки Стебли Корни	+++++ +++++ +++++ +	11 10 8 8	0,46;0,54; 0,67 0,69 0,66 0,59	- - + +
53	Gentianaceae <i>Gentiana gelida</i> Bieb.	Горийский р-н.с. Бошупара, 2016	Листья Цветки Стебли	+++++ +++++ +++	9 8 5	0,40;0,58; 0,76 0,17; 0,58 -	- + ++
54	<i>G.septemfida</i> Pall.	Бакуриани, окр. Дидвели, 2016	Листья Цветки Стебли	+++++ +++++ ++++	9 8 4	0,56; 0,52;0,60;0,68 -	+ +++ ++
55	<i>Swertia iberica</i> Fisch. & C.A.Mey	Бакуриани, окр. Дидвели, 2016	Листья Цветки Стебли	++++ +++ +	7 4 5	0,51 0,51 -	- + +
56	Helleboraceae <i>Consolida orientalis</i> (J.Gay) Schrodinger.	окр. Коджори, 2016	Листья Цветки Стебли Корни	+++ + - -	4 3 1 -	0,29; 0,60 - - -	- +++ ++ +++
57	<i>C. divaricata</i> (Ledeb.) Schrodinger.	Окр. с. Дигори, 2016	Плоды Цветки Стебли	- + +	- 2 2	- - -	- - ++
58	Hydrangeaceae <i>Philadelphus caucasicus</i> Koehne.	На дороге Чаргали, 2016	Листья Плоды Стебли	++++ + +	4 5 2	0,61;0,68;0,73; 0,80 - -	+? - -

59	Hyacinthaceae <i>Scilla siberica</i> Haw.	Цавкиси 2017	Лист,ст.	+++++	2	0,30	-
			Цветки	+++++	5	-	-
			Стружки	-	4	-	+
60	Labiatae <i>Betonica officinalis</i> L.	Алгет-ское водохранилище, 2016	Плоды	+++	3	0,16;0,20;0,33;0,64	+
			Цветки	++	2	0,59; 0,72	++
			Стебли	++	5	-	+
61	<i>B. officinalis</i> L.	Манглиси, Тонети, 2016	Листья	+++++	6	0,12;0,18;0,41; 0,58	+
			Цветки	+++++	5	0,12; 0,24	-
			Стебли	++++	3	0,28	+
			Корни	+	3	0,32; 0,53;0,74	+
62	<i>B. officinalis</i> L.	Хребет Цивгомбори, 2017	Листья	+++++	4	0,17;0,24;0,38	+
			Цветки	+++++	6	-	следы
			Стебли	+++++	5	-	++
63	<i>B. orientalis</i> L.	окр. оз. Паравани, 2017	Листья	+++++	8	0,07;0,23;0,43 0,84	+
			Цветки	+++++	12	0,07;0,09;0,84;0,91 0,09; 0,40	+++
			Стебли	++++	6	-	+++
64	<i>Calamintha</i> sp.	Бакуриани окр. Дидвели 2016	Листья	+++++	8	0,67; 0,74	+
			Цветки	+++++	8	0,69; 0,76	++
			Стебли	+++	7	-	+
			Корни	-	3	-	++
65	<i>Glechoma hederacea</i> L.	Горийский р-н.с .Хидистави, 2016	Листья	+	1	-	++
			Стебли	+++++	7	0,18,0,22;0,47	++
66	<i>G. hederacea</i> L.	Горийский р-н.с .Хидистави, 2017	Надз. часть	+++++	13	-	+
67	<i>Mentha arvensis</i> L.	Горийский р-н, окр. р. Тана, 2016	Листья	+++++	5	0,49	++
			Цветки	+++++	5	0,46;0,54;0,68	+
			Стебли	+	3	-	-
68	<i>Nepeta cataria</i> L.	окр. Цавкиси, 2016	Листья	++++	4	0,22;0,34;0,48; 0,65	-
			Стебли	+++	4	0,18;0,32;0,46	+
69	<i>N. mussini</i> Spreng.	Тетрицкаро, окр. Храмгеси-2, 2017	Листья	+++++	10	0,12; 0,30	+
			Цветки	+++++	6	-	++
			Стебли	-	7	-	+++
			Корни	-	3	-	-
70	<i>Origanum vulgare</i> L.	Горийский р-н, ущ. Р. Тана, 2016	Листья, Цветки	+++	7	0,29; 0,43	-
			Стебли	+	2	-	++
71	<i>Salvia glutinosa</i> L.	лев. стор. дороги Тианети, 2016	Листья	++	3	0,29;0,43;0,87	-
			Стебли	-	1	-	++
72	<i>S. nemorosa</i> L.	Тетрицкаро, окр. Храмгеси-2, 2017	Листья	+++++	9	0,17;0,46;0,60	+?
			Цветки	+	8	0,57; 0,97	++
			Стебли	++	6	-	++
			Корни	-	2	-	+
73	<i>Satureja laxiflora</i> C. Koch.	ущ. Цавкиси, 2016	Лист,ст.	+++++	6	0,08;0,16;0,25; 0,58	+
			Корни	+?	3	0,78; 0,86	+
74	Linaceae <i>Linum hypericifolium</i> Salisb.	Хребет Цивгомбори, 2017	Листья	-	4	-	-
			Цветки	+++	6	-	+
			Стебли	++	2	-	++
			Корни	-	2	-	++

75	Loranthaceae <i>Viscum album</i> L.	окр. Дидго- ри, 2017	Листья Стебли	++ -	3 4	0,33 0,42	- +++
76	Malvaceae <i>Alcea rugosa</i> Alef.	Горийский р-н.с .Хиди- стави 2016	Листья Плоды Стебли	+++ - +	8 - 4	- - -	++ - +++
77	<i>A. rugosa</i> Alef.	Горийский р-н.с .Хиди- стави, 2017	Листья Плоды Стебли Корни	++ + - -	7 6 3 9	0,11 0,92 - -	+ ++ ++ ++
78	<i>Lavatera</i> <i>thuringiaca</i> L.	Хребет Цивгомбо- ри, 2017	Листья Цветки Плоды Стебли	++++ ++++ ++++ +	12 16 13 7	0,00; 0,13 0,00;0,86;0,91 0,00;0,24;0,90 0,00	++ + ++ +++
79	Oleacea <i>Osmanthus</i> <i>fragrans</i> Lour	Зугдидский ботаничес- кий сад,2016	Листья Стебли	++++ +?	3 2	0,26; 0,62 0,71	+++ +++
80	Onagraceae <i>Circea lutetiana</i> L.	Окр. Зеда- зени, 2017	Листья Цветки Стебли Корни	+ ++ + -	6 7 4 4	0,43; 0,73 0,51 - -	- - + ++
81	<i>Chamaeneriu</i> <i>angustifolium</i> (L.) Scop.	Окр. Дидго- ри, 2016	Листья Цветки Стебли	++++ ++++ ++++	3 5 7	0,12 0,12;0,65;0,81 0,11; 0,64	+ - +
82	<i>Ch. angustifo-</i> <i>lium</i> (L.)Scop.	Оз.Парава ни, 2016	Листья Цветки Стебли	++++ ++++ ++++	4 4 3	0,12 0,12;0,57;0,65; 0,81 0,12	+ - +
83	<i>Epilobium</i> <i>hirsutum</i> L.	ущ. Атени, 2016	Листья Цветки Стебли Корни	++++ ++++ +++ -	3 2 5 2	0,11; 0,13 0,11; 0,13 0,13 -	- + + +
84	Primulaceae <i>Anagallis foe-</i> <i>mina</i> Mill.	Окр. Давид- Гареджи, 2016	Листья Плоды Стебли Корни	+++ ++++ +++ -	4 4 5 -	0,47; 0,63 0,36;0,55;0,68 0,56; 0,67 -	+ + + -
85	<i>Primula macro-</i> <i>calyx</i> Bunge.	Тбилиси, окр.Глдани, 2016	Листья Цветки Стебли	++++ ++++ +++	4 3 4	0,26; 0,30 0,15; 0,22; 0,28;0,21; 0,30	+ + +
86	Polygonaceae <i>Atraphaxis cau-</i> <i>casica</i> (Hoffm.) Pavl.	Тбилиси, окр. Коджо- ри, 2017	Листья Цветки с Семенной Стебли	++++ ++++ +?	11 8 4	0,52; 0,73; 0,830,72; 0,81 0,21; 0,30	- - +++
87	<i>Polygonum</i> <i>aviculare</i> L.	Горийский р-н.с. Хиди- стави 2017	Надз. часть	++++	5	0,55;0,69;0,81;0,90	+
88	<i>P.carneum</i> C.Koch.	Окр. оз. Паравани, 2017	Листья Цветки Стебли Корни	++++ ++++ ++++ -	11 5 6 3	0,56; 0,64; 0,76 0,79; 0,87 0,24	- + ++ +
89	<i>P.convulvulus</i> L.	Хребет Цив гомбори, 2017	Целое растение	++++	6	0,59; 0,73	следы
90	Rosaceae <i>Agrimonia</i> <i>eupatoria</i> L	окр.ущ.Цав- киси, 2016	Листья Стебли	+++ ++	7 5	0,32;0,68;0,79; 0,93 0,12;0,16;0,83	- -

91	<i>Filipendula ulmaria</i> (L.) Maxim.	Бақуриани, окр. Дидвेलи, 2016	Листья Цветки Стебли	+++++ +++++ +++	9 12 6	0,60; 0,65 0,58;0,69;0,80 0,12;0,16;0,53	+ ++ ++
92	<i>F. ulmaria</i> (L.) Maxim	Бақуриани, окр. Бақурцихе, 2016	Листья Цветки Стебли	+++ ++ +	7 3 6	- 0,60 -	- + -
93	<i>F. ulmaria</i> (L.) Maxim	Бақуриани окр. Дидвेलи, 2017	Листья Плоды, семена Стебли	+++++ ++ ++++	8 9 11	0,31 0,37 0,30	- ++ +
94	<i>Potentilla anserine</i> L.	Ниноцминда, окр. Паравани, 2016	Листья Стебли	+++ ++	3 2	- -	+ +
95	<i>Rubus ideus</i> L.	Бақуриани, окр. Дидвेलи, 2017	Листья Стебли	+++++ ++	10 7	0,31; 0,61 -	+ +++
96	Rubiaceae <i>Crucianella angustifolia</i> L.	Патара Дидгори, 2017	Плоды, семена Стебли Корни	++ +++ -	6 5 -	0,54 0,54; 0,68 -	- +++ -
97	<i>Galium cruciatum</i> (L.) Scop.	уш.Цавкиси, 2016	Листья Стебли	+++++ +	5 3	0,31 0,60	++ ++
98	Rutaceae <i>Dictamnus caucasicus</i> (Fisch.& C.A.Mey.) Grossh.	Асурети, 2017	Листья Цветки Стебли Корни	+++++ +++++ +++++ -	9 10 6 3	0,49;0,61;0,68 9,49; 0,61 0,47 -	+ + ++ ++
99	Santalaceae <i>Thesium arvense</i> Horvatuszky	Патара Дидгори 2016	Стебли Корни	+++ -	2 2	- -	+ -
100	Scrophulariaceae <i>Euphrasia stricta</i> Hoss.	Бақуриани окр. Дидвेलи, 2017	Цветки, семена Стебли	+++ +++	9 12	0,04 0,04	- +
101	<i>Linaria caucasicana</i> Kem.-Nath.	Окр. Бақуриани 2016	Листья, плоды Цветки Стебли Корни	+ ++ ++ +?	1 2 1 2	- - - -	+ + +++ +
102	<i>Melampyrum arvense</i> L.	Тбилиси, окр. Цодорети, 2016	Листья Цветки Стебли	+++++ + +	10 5 5	0,26;0,47;0,54; 0,63;0,78 0,24;0,42;0,54; 0,62 0,25, 0,54	++ +++ ++
103	<i>Pedicularis comosa</i> L.	Тбилиси, окр.Цкнети, 2017	Лист,стеб. Цветки Корни	+++ +++ -	9 9 3	0,04; 0,14 0,04;0,14;0,85 -	- + +
104	<i>Rhynchocorys orientalis</i> (L.).Benth.	Окр. Норио 2016	Листья Плоды Стебли Корни	+? ++ ++ -	6 2 5 -	0,32; 0,57 - 0,30 -	+ + + -
105	Solanaceae <i>Physalis alkekengi</i> L.	Горийский р-н, с.Хидистави (собр. 28.VI) 2016	Листья Цветки Плоды Стебли	++ +? - +?	3 2 - 1	0,68 - - -	- - + -

106	Ph. alkekengi L.	Горийский р-н, с.Хидистави, (собр. 29.X)2016	Листья Цветки Стебли	+++ +? +?	4 3 2	0,68; 0,71 - -	++ - -
107	Solanum pseudopersicum Pojark.	прав. стор. дороги Тианети, 2016	Листья Плоды Стебли Корни	++ +? + -	7 - 3 6	0,70 - - -	+ + + ++
108	Valerianaceae Centranthus longifolius Stev.	Асурети, 2016	Листья Цветки Стебли	+++++ +++++ +++++	5 3 4	0,43;0,59,0,78 0,61 0,59; 0,76	+ + +++
109	Valeriana alliariaefolia Adams	Бакуриани окр. Дидвели, 2016	Листья Цветки Стебли Корни	+++ ++ + -	5 5 6 5	- 0,63 - -	+ + +++ +++
110	Verbenaceae Verbena officinalis L.	Тианети, по дороге Чаргали, 2016	Листья Семена Стебли	+++ ++ -	8 2 -	0,07;0,13;0,19; 0,51 0,13; 0,93 -	+ - +
111	Umbelliferae Astrantia maxima Pall.	Тбилиси, левый берег р. Вере, 2017	Листья Цветки Стебли	+++++ +++++ -	5 13 6	0,27;0,34; 0,65; 0,90 0,11;0,14; -	- - -
112	Bupleurum wittmannii Stev.	Давид Гареджи, 2016	Листья Цветки, Плоды Стебли Корни	+++++ +++++ +++++ -	7 5 3 -	0,19;0,41;0,47; 0,58; 0,72 0,51; 0,60 0,66 -	- - +++ +++
113	B. wittmannii Stev.	Давид Гареджи, 2016	Листья Цветки, Плоды Стебли Корни	+++++ +++++ +++++ -	7 5 3 -	0,19;0,41;0,47; 0,58; 0,72 0,51; 0,60 0,66 -	- - +++ +++
114	Laser trilobum (L.)Borkh 2017	Окр. Норио, 2017	Листья Стебли	++++ +	8 4	0,48; 0,62; 0,91 -	+++ ++
115	Pimpinella rhodantha Boiss.	Хребет Цивгомбори, 2017	Листья Цветки Стебли Корни	++++ +++++ + -	8 9 7 3	0,12; 0,33; 0,44; 0,55 0,22;0,46;0,61 - - -	- ++ + +++
116	Smiranium perfoliatum L.	Тбилиси, окр. Патара Дидгори, 2017	Листья Стебли Семена корни	+++++ +++ +? -	9 7 3 -	0,60; 0,72 0,69 - -	- +++ +++ ++
117	S. perfoliatum L.	Окр.Патара Дидгори, 2016	Листья Стебли Корни	+++ +? +?	1 1 1	0,86 - -	+++ +++ +++

Циклоартановые тритерпеноиды выявлены в представителях семейств *Asteraceae*, *Apiaceae*, *Anacardiaceae*, *Convolvulaceae*, *Fabaceae*, *Geraniaceae*, *Helleboraceae*, *Malvaceae*, *Oleaceae*, *Polygonaceae*, *Scrophulariaceae*, *Umbelliferae*, *Vallerianaceae* (таблица).

Известно, что фармакологически активные препараты вида рода *Geranium* обладают антиоксидантной, гепатопр-

текторной, неиродегенеративной, гастроинтестинальной, бактериостатической, фунгицидной активностью [1]. Сотрудниками Института фармакохимии установлено, что этилацетатная сумма из надземной части *Geranium pusillum* обладает антиоксидантной, бактерио- и фунгицидной активностью [1]. Растение *Polygonum carneum*, произрастающее во флоре Грузии, на содержание флавоноидов не про-

анализировано. Отвар растения применяется при острых и хронических заболеваниях кишечника, тонзиллитах, гингивитах [7].

Geranium ibericum и *Polygonum carneum* ввиду интересного флавоноидного состава подвергались сравнительно глубокому химическому анализу. Разделение полученных сумм из этих видов на индивидуальные компоненты осуществляли классическими методами [2]. Из надземных частей обеих растений выделили вещества: 1 и 2 (*G. ibericum*); вещества 3 и 4 (*P. carneum*).

Для идентификации изолированных веществ использовали цветные реакции, физико-химические константы как самих веществ, так и продуктов их кислотного и щелочного гидролиза, данные ИК, УФ-спектроскопии [2].

Вещество 1 - состава $C_{21}H_{20}O_{12}$, желтого цвета игольчатые кристаллы с т.пл. 236-238°C (из метанола). УФ-спектр: λ_{max} , метанол, нм: 360, 262; ИК-спектр: ν_{max} , КВг, cm^{-1} : 3300(OH), 1665(C=O), 1605, 1665, 1515 (C=C). На основании полученных данных и их сравнения с аутентичным образцом гиперина вещество 1 идентифицировали как кверцетин-3-0- β -D-галактопиранозид (гиперин) [2].

Вещество 2 - состава $C_{15}H_{10}O_7$, яркожелтые кристаллы с т.пл. 310-312°C (из метанола). УФ-спектр: λ_{max} , метанол, нм: 372, 257; ИК-спектр: ν_{max} , КВг, cm^{-1} : 3385, 3300(OH), 1665(C=O), 1565 и 1516 (C=C). На основании полученных данных и их сравнения с аутентичным образцом кверцетина вещество 2 идентифицировали как 3,5,7',3'.4'-тригидроксифлаво-н (кверцетин) [2].

Вещество 3 - состава $C_{27}H_{30}O_{16}$, желто-зеленоватого цвета кристаллы с т.пл. 189-191°C. УФ-спектр: λ_{max} , метанол, нм: 372, 257; ИК-спектр: ν_{max} , КВг, cm^{-1} : 3450(OH), 1650(C=O), 1610, 1585, 1520 (C=C). Вещество 3 воздействием кислоты расщепляется на агликон-кверцетин, D-глюкозу и L-рамнозу. В результате анализа и сравнения их с аутентичным образцом рутина позволили идентифицировать вещество 3 как кверцетин-3-0-рутинозид или рутин [2].

Вещество 4, выделенное из *Polygonum carneum*, оказалось идентичным веществу 2 (кверцетин), изолированному из *G. ibericum*.

Вещества 1, 2 из *G. ibericum* и вещества 3 и 4 *P. carneum* флоры Грузии выделены впервые. Эти виды можно применять в качестве сырья кверцетина и рутина.

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SUMMARY

RESULTS OF THE PRELIMINARY STUDY OF PLANTS OF GEORGIAN FLORA FOR THE CONTENT OF FLAVONOIDS AND TRITERPENOIDS

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The aim of the study was a preliminary study of plant samples collected by targeted expeditions of the department of pharmacobotany (Department of Phytochemistry) of the IG Kutateladze Institute of Pharmacochemistry for the period 2016-2017. In total, 341 objects from (106 various) plants belonging to 36 families and 93 genus were analyzed. The objects were obtained polar and non-polar fractions; flavonoids and triterpenoids were found in them qualitatively. To characterize used P/CH and TLC analysis in various solvent systems.

Flavonoids were isolated from *Geranium ibericum* and *Polygonum carneum*, which were identified as quercetin-3-0- β -D-galactopyranoside-hyperin (*G. ibericum*), 3 5, 7 ' , 3'.4' pentahydroxy-flavone – quercetin (*G. ibericum*, *P. carneum*) and quercetin-3-0-rutinozide – rutin (*P. carneum*).

G. ibericum and *P.carneum* flora of Georgia are found and studied for the first time.

Representatives of the family *Asteraceae*, *Fabaceae*, *Helleboraceae*, *Polygonaceae*, *Scrophulariaceae* deserve a particular interest in the content of triterpenoids.

Keywords: flavonoids, cycloartanes, rutin, hyperin, quercetin.

РЕЗЮМЕ

РЕЗУЛЬТАТЫ ПРЕДВАРИТЕЛЬНОГО ИССЛЕДОВАНИЯ РАСТЕНИЙ ФЛОРЫ ГРУЗИИ
НА СОДЕРЖАНИЕ ФЛАВОНОИДОВ И ТРИТЕРПЕНОИДОВ

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Целью исследования являлось предварительное изучение видов растений, собранных целевыми экспедициями направлением фармакоботаники Института фармакохимии им. И. Г. Кутателадзе за период 2016-2017 гг. Проанализирован 341 объект из 117 (106 разных) растений, относящихся к 36 семействам и 93 родам. Из растений получали полярные и неполярные фракции. Для обнаружения флавоноидов и тритерпеноидов применяли качественные реакции, Б/Х и ТСХ анализы в разных системах растворителей.

Среди анализируемых объектов особенно выделялись виды семейств *Fabaceae*, *Asteraceae*, *Geraniaceae*, *Polygonaceae*, *Labiatae*, *Rosaceae*.

Глубоко изучены виды *Geranium ibericum* и *Polygonum carneum*; из них выделены флавоноиды, которые с учетом физико-химических свойств, данных ИК и УФ-спектров и продуктов химического превращения идентифицированы как кверцетин-3-0-β-D-галактопиранозид - гиперин (*G. ibericum*), 3,5,7',3'.4'-пентагидрокси-флавоноид - кверцетин (*G. ibericum*, *P. carneum*) и кверцетин-3-0-рутинозид - рутин (*P. carneum*). Флавоноиды *G. ibericum* и *P. carneum* флоры Грузии изучены впервые.

По содержанию тритерпеноидов особенно выделялись представители семейств *Asteraceae*, *Fabaceae*, *Helleboraceae*, *Polygonaceae*, *Scrophulariaceae*.

რეზიუმე

საქართველოს ფლორის მცენარეთა წინასწარი გამოკვლევა ფლავონოიდების და ტრიტერპენების შემცველობაზე

ქ.შალაშვილი, მ.სუთიაშვილი, თ.საღარეიშვილი, ჯ.ანელი, მ.ალანია

თბილისის სახელმწიფო სამედიცინო უნივერსიტეტი,
ი. ქუთათელაძის ფარმაკოქიმიის ინსტიტუტი, საქართველო

კვლევის მიზანს წარმოადგენდა ფარმაკოქიმიის ინსტიტუტის ფარმაკობოტანიკის დეპარტამენტის მიზნობრივი ექსპედიციების მიერ 2016-2017 წლებში შეგროვილი მცენარეთა ნიმუშების წინასწარი შესწავლა ფლავონოიდების და ტრიტერპენების შემცველობაზე. გაანალიზებულია 36 ოჯახის და 93 გვარის 117 (106 სხვადასხვა) მცენარის 341 ნიმუში. ობიექტებიდან მიღებულ პოლარულ და არაპოლარულ ფრაქციებში გამოვლინდა ფლავონოიდების და ტრიტერპენოიდების მნიშვნელოვანი შემცველობა. დასახასიათებლად გამოყენებულია თვისობრივი რეაქციები, ქ/ქ და თფქ ანალიზი სხვადასხვა გამსხნელთა სისტემაში.

გაანალიზებული ობიექტებიდან განსაკუთრებით გამოირჩევა ოჯახები *Fabaceae*, *Asteraceae*, *Geraniaceae*, *Polygoniaceae*, *Labiatae*, *Rosaceae*. ფლავონოიდების მნიშვნელოვანი შემცველობით გამოირჩეულია *Geranium*

ibericum და *Polygonum carneum* შედარებით ღრმად იყო შესწავლილი. მათგან იზოლირებული ფლავონოიდები, ფიზიკურ-ქიმიური თვისებების, ინფრაწითელი და ულტრაიისფერი-სპექტრული მონაცემების და ქიმიური გარდაქმნის პროდუქტების შესწავლის შედეგად იდენტიფიცირებული არიან, როგორც კვერცეტინ-3-0-β-D-გალაქტოპირანოზიდ-ჰიპერინი (*G. ibericum*), 3,5,7',3'.4'-პენტაჰიდროქსი-ფლავონი - კვერცეტინი (*G. ibericum*, *P. Carneum*) და კვერცეტინ-3-0-რუტინოზიდ - რუტინი (*P. Carneum*).

საქართველოს ფლორის *G. ibericum* და *P. carneum* პირველადია შესწავლილი.

ტრიტერპენების შემცველობის მხრივ განსაკუთრებულ ინტერესს იწვევს *Asteraceae*, *Fabaceae*, *Helleboraceae*, *Polygonaceae*, *Scrophulariaceae*-ს ოჯახების წარმომადგენლები.

АРТЕРИАЛЬНАЯ ГИПЕРТЕНЗИЯ У РАБОЧИХ УРАНОПЕРЕРАБАТЫВАЮЩЕГО ПРЕДПРИЯТИЯ КАЗАХСТАНА: РАСПРОСТРАНЕННОСТЬ, ОТНОСИТЕЛЬНЫЕ РИСКИ И ПРЕДИКТОРЫ РАЗВИТИЯ

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Республика Казахстан занимает одно из ведущих мест в мире по добыче и переработке урановой руды. Выполнение основных технологических операций на предприятиях атомной промышленности сопровождается перманентным воздействием на персонал комбинированных радиационно-химических факторов: внешнего и внутреннего облучения в малых дозах, пыли, газов, раздражителей, токсических соединений урана, психоэмоциональных перегрузок [6,10]. По мнению ряда авторов, сочетание различных антропогенных факторов физической, в том числе радиационных, и химической природы может увеличивать риск хронических заболеваний внутренних органов. При этом радиационный фактор может и не играть определяющую роль, но выступать в качестве агента, потенцирующего воздействие традиционных предикторов хронических заболеваний внутренних органов, в том числе артериальной гипертензии [2,3,7,14].

Установлено, что эффект низкоуровневого облучения может многократно усиливаться в комбинации с другими физическими и химическими неблагоприятными производственными факторами. Имеющиеся в доступной литературе сведения о распространенности артериальной гипертензии (АГ) среди работников предприятий атомной индустрии малочисленны, фрагментарны и в ряде случаев противоречивы [1,8,9,15,16,21].

Целью исследования явилось изучение распространенности, относительных рисков и предикторов развития АГ у рабочих ураноперерабатывающего предприятия, подвергающихся в процессе профессиональной деятельности воздействию ионизирующего излучения в диапазоне «малых» доз.

Материал и методы. Изучены распространенность (Pr), относительные грубые (RR) и стандартизованные (SRR) риски АГ у 809 рабочих мужского пола Гидрометаллургического завода (ГМЗ) г. Степногорска, являющегося крупнейшим в Средне-Азиатском регионе и Казахстане предприятием по производству технической закиси-окси урана. Распространенность АГ изучалась с помощью открытого сплошного поперечного (кросс-секционного) сравнительного исследования рабочих ГМЗ, охватившего 96,9% персонала основных производственных цехов предприятия. По данным службы радиационной безопасности ГМЗ, регистрируемая радиационная нагрузка на персонал в течение ряда лет была равномерна. Средняя индивидуальная доза внутреннего и внешнего облучения за 2013-2018 гг. составила 6,82 мЗв/год. Группу сравнения составили 696 работников Степногорского подшипникового завода (СПЗ), расположенного на расстоянии 10 км от ГМЗ. Выбор группы сравнения обусловлен одинаковыми с экспонируемой группой социально-бытовыми, природно-климатическими условиями. Все рабочие экспонируемой группы и группы сравнения проживали в г. Степногорске и имели одинаковый уровень облучения от естественных источников. Группа сравнения отличалась от экспонируемой, отсутствием воздействия радиационно-химических факторов, присущих ураноперерабатывающему производству.

В качестве меры сравнения рассчитывали RR и SRR. 95% доверительный интервал (CL) для RR и SRR рассчитывали по методу Катца. Для сравнения независимых выборок по би-

нарному признаку (распространенность) дополнительно, для оценки статистической значимости различий, проводили анализ четырехпольной таблицы сопряженности с использованием критерия χ^2 [11,13].

Для изучения взаимосвязи между изучаемыми количественными и качественными переменными использовали метод нелинейной множественной бинарной логистической регрессии. Построено две модели - для ГМЗ и СПЗ. Оценку полученных в результате построения моделей весовых β -коэффициентов проводили с помощью прямого пошагового алгоритма (прогрессивная селекция). Силу связи каждого предиктора (фактора риска) с распространенностью АГ оценивали по отношению шансов (ОШ), рассчитанных на основании полученных β -коэффициентов [13].

Для изучения взаимосвязи между распространенностью и факторами риска (ФР) развития АГ оценивали наличие следующих, наиболее значимых ФР (предикторов) развития АГ: возраст, отягощенная наследственность, ожирение, метаболический синдром, употребление алкоголя, пристрастие к соленой пище, низкая физическая активность, психоэмоциональное перенапряжение, контакт с источниками ионизирующего излучения (ИИ) и вредными химическими веществами [3,9,23].

Для характеристики антропометрических данных использовали индекс массы тела (ИМТ). О наличии метаболического синдрома (МС) судили на основании критериев International Diabetes Federation [19].

Расчет употребления этанола (г/неделя) проводили в соответствии с классификацией В.В. Дунаевского и В.Д. Стяжкина [4]. По количеству употребляемого этанола сформированы группы абстинентов, случайно, умеренно, систематически и привычно пьющих работников. Для оценки психоэмоционального напряжения использована методика диагностики самооценки уровня тревожности Ч.Д. Спилберга в адаптации Ю.Л. Ханина [17]. Методика позволяет дифференцированно измерять тревожность как личностное свойство (уровень личностной тревожности), и как личностное состояние (уровень ситуативной тревожности). Оценку уровня реактивной (РТ) и личной (ЛТ) тревожности проводили однотипно: ≤ 30 баллов – низкая, 31-45 – умеренная, ≥ 46 – высокая.

Результаты и их обсуждение. Pr АГ изучена среди 1505 рабочих ГМЗ и СПЗ. Анализ показателей Pr выявил высокую пораженность АГ персонала ураноопасного предприятия (таблица 1).

Относительный риск АГ в экспонируемой группе был избыточным (RR=1,8; 95% CL 1,4-2,3). Выявленная высокая распространенность АГ в экспонируемой группе совпадает с данными ряда исследователей. Так, риск АГ (относительный шанс 1,6; 95% CL 1,1-2,4) повышен у рабочих Сибирского химического комбината, непосредственно контактирующих с источниками ИИ в сравнении с лицами без такового [7,14]. В тоже время, в результате анализа медико-дозиметрического регистра Канады, Великобритании и производственного объединения «Маяк» (Россия) исследователи не смогли констатировать факт положительной связи между радиационной дозой у персонала и сердечно-сосудистой заболеваемостью [20,22].

Таблица 1. Распространенность и грубый риск АГ у рабочих ГМЗ и СПЗ

ГМЗ		СПЗ		RR ГМЗ vs СПЗ (95% CL)
n	Pr, % (95% CL)	n	Pr, % (95% CL)	
193	23,9 (21,0-26,8)	91	13,1 (10,3-15,9)	1,8 (1,4-2,3)

Анализ относительных рисков АГ в зависимости от возраста у персонала ГМЗ и СПЗ мужского пола показал, что начиная с 30 лет риск болеть АГ среди рабочих ГМЗ был в 1,5 раза выше, чем среди рабочих СПЗ; в возрастных группах 40-49 лет и 50-59 лет относительный риск повышался до 1,98 и 2,2, соответственно (рис.).

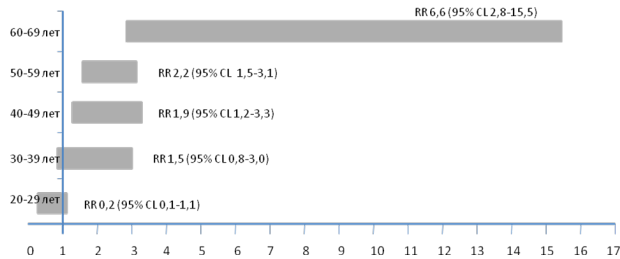


Рис. 95% доверительные интервалы относительных рисков (RR) АГ в зависимости от возраста у работников ГМЗ vs СПЗ

Относительный риск АГ в 60-69-летней возрастной группе значительно превышал RR в других возрастных группах, однако имел широкий 95% CL, что снижало ценность количественной оценки RR.

Исследования по изучению эпидемиологии сердечно-сосудистых заболеваний (ССЗ) и их ФР среди рабочих промышленных предприятий показали, что условия профессиональной деятельности, в том числе воздействие ИИ, влияя на уровни отдельных предикторов (ФР), имеют определенное значение в формировании ССЗ [2,5,12].

Результаты сравнения частоты распространения изучаемых ФР развития АГ среди персонала ГМЗ и СПЗ приведены в таблице 2. Обращает внимание значительная распространенность ЛТ высокой степени среди 58,2% рабочих ГМЗ (95%CL 54,8-61,6), превосходящая аналогичный показатель среди рабочих СПЗ в 21,6 раза ($p < 0,0001$). Показатели РТ высокой степени среди рабочих ГМЗ также были выше, чем в группе сравнения (RR 4,9 (95% CL 2,9-8,4; $\chi^2=33,4; p < 0,0001$)). Известно, что работа во вредных и опасных условиях сопровождается большой эмоциональной нагрузкой, связанной с чувственной неопределенностью опасности и субъективной неопределенностью прогноза, между тем установлено, что длительный хронический стресс ведёт к развитию АГ [5,9,14,24].

Таблица 2. Распространенность и относительный риск предикторов АГ у рабочих мужского пола

Предикторы	ГМЗ		СПЗ		RR (95% CL)	χ^2	p
	n	Pr, % (95% CL)	n	Pr, % (95% CL)			
Наследственность	136	16,8 (14,2-19,4)	104	14,9 (12,3-17,5)	1,1 (0,9-1,4)	0,84	0,359
Ожирение	74	9,1 (7,1-11,1)	61	8,8 (6,7-10,9)	1,0 (0,7-1,4)	0,03	0,866
– Ожирение 1 ст.	41	5,1 (3,6-6,6)	40	5,7 (4,0-7,4)	0,9 (0,6-1,4)	0,22	0,640
– Ожирение 2 ст.	26	3,2 (2,0-4,4)	18	2,6 (1,4-3,8)	1,2 (0,7-2,2)	0,32	0,571
– Ожирение 3 ст.	7	0,9 (0,2-1,6)	3	0,4 (-0,1-0,9)	2,3 (0,6-8,9)	0,51	0,474
Метаболический синдром	11	1,4 (0,6-2,2)	15	2,2 (1,1-3,3)	0,6 (0,3-1,3)	0,97	0,326
Употребление алкоголя	687	84,9 (82,4-87,4)	532	76,4 (73,2-79,6)	1,1 (1,0-1,2)	16,94	<0,0001
– Случайно пьющие	243	30,0 (26,8-33,2)	192	27,6 (24,3-30,9)	1,1 (0,9-1,3)	0,98	0,322
– Умеренно пьющие	282	34,9 (31,6-38,2)	188	27,0 (23,7-30,3)	1,3 (1,1-1,5)	10,36	0,001
– Систематически пьющие	162	20,0 (17,2-22,8)	152	21,8 (18,7-24,9)	0,9 (0,7-1,1)	0,64	0,424
Личностная тревожность	-	-	-	-	-	-	-
– Низкая	3	0,4 (-0,03-0,8)	117	16,8 (14,0-19,6)	0,02 (0,008-0,075)	135,6	<0,0001
– Умеренная	335	41,4 (38,0-44,8)	560	80,5 (77,6-83,4)	0,51 (0,47-0,56)	235,1	<0,0001
– Высокая	471	58,2 (54,8-61,6)	19	2,7 (1,5-3,9)	21,6 (13,8-33,8)	522,1	<0,0001
Реактивная тревожность	-	-	-	-	-	-	-
– Низкая	40	4,9 (3,4-6,4)	351	50,4 (46,7-54,1)	0,1 (0,07-0,14)	400,1	<0,0001
– Умеренная	682	84,3 (81,8-86,8)	330	47,4 (43,7-51,1)	1,8 (1,7-2,0)	229,5	<0,0001
– Высокая	87	10,8 (8,7-12,9)	15	2,2 (1,1-3,3)	4,9 (2,9-8,4)	33,4	<0,0001
Пристрастие к соленой пище	252	31,1 (27,9-34,3)	180	25,9 (22,6-29,2)	1,2 (1,0-1,4)	4,86	0,027
Низкая физическая активность	64	7,9 (6,0-9,8)	52	7,5 (5,5-9,5)	1,1 (0,8-1,6)	0,05	0,824

Распространенность употребления алкоголя на изучаемых производствах была достаточно высокой, составляя 84,9% на ГМЗ и 76,4% на СПЗ. Данные эпидемиологических исследований свидетельствуют, что риск развития АГ повышается только при систематическом употреблении алкоголя (112 г. в неделю или 140 мл в неделю чистого этанола у мужчин). Между тем, распространенность систематического употребления алкоголя между сравниваемыми предприятиями была статистически незначимой. Из таблицы 2 следует, что достоверных различий в показателях превалентности злоупотребления поваренной солью, низкой физической активности, ожирения, метаболического синдрома и отягощенной по АГ наследственности не выявлено.

Анализ данных литературы свидетельствует о существовании экзогенных факторов – производственных вредных, в том числе ИИ, которые могут быть отнесены к факторам риска развития АГ [6,7,14,18]. В этой связи в качестве возможного предиктора АГ рассматривается показатель суммарной дозы облучения (СДО).

Так как одномерные сравнения групп в принципе не могут реализовать модель совокупного влияния многих факторов на развитие АГ, решено прибегнуть к помощи многомерных методов. Оценка взаимосвязи АГ и комплекса предикторов проводилась путём построения уравнений нелинейной множественной бинарной логистической регрессии. Построено две модели - для ГМЗ и СПЗ, связывающие распространенность АГ с предикторами заболевания. Основной целью использования данного статистического метода явилось определение вероятности АГ в зависимости от наличия и сочетания её предикторов, т.е. ранняя диагностика заболевания.

В программе SPSS 13.0 построены следующие уравнения логистической регрессии (модели):

$$Prob_{ГМЗ} = \frac{1}{1 + e^{-1,156 + 2,609n + 0,514s + 0,053m - 0,381t - 0,693a}} ; (1)$$

$$Prob_{СПЗ} = \frac{1}{1 + e^{-2,099 + 2,221n + 0,15t + 0,003m - 1,56t - 0,543a}} (2), \text{ где}$$

Prob_{ГМЗ} - вероятность АГ у рабочих ГМЗ; Prob_{СПЗ} - вероятность АГ у рабочих СПЗ; e - экспонента (2,71828); n - наследственность (0 или 1); s - СДО (мЗв); m - стаж (лет); t - ИМТ (кг/м²); a - употребление алкоголя (г/нед).

По результатам проведенного множественного пошагового корреляционно-регрессионного анализа выявлено пять значимых независимых факторов, влияющих на распространенность АГ: наследственность, СДО (для СПЗ – продолжительность трудового стажа), ИМТ, ЛТ, употребление алкоголя (таблица 3).

Поскольку на СПЗ отсутствует техногенное ИИ, нами введен условный показатель СДО для каждого из работников СПЗ, равный 1 мЗв, что позволило провести расчет ОШ АГ для персонала ГМЗ.

Силу связи каждого предиктора с распространенностью АГ оценивали по β-коэффициентам.

При множественном бинарном логистическом анализе β-коэффициент - это натуральный логарифм ОШ, в связи с чем расчет ОШ для связи предиктора с заболеванием производили проводя логит-преобразование - экспонента («антилогарифм») «β».

В отличие от отношения шансов, рассчитанного с использованием стандартной формулы, в данном случае отношение шансов указывает на изменение шанса развития заболевания при изменении на одну единицу независимой переменной (предиктора).

Наиболее весомым предиктором АГ на обоих предприятиях являлась наследственная предрасположенность. Из таблицы 3 явствует, что влияние наследственной предрасположенности на Рг АГ у рабочих ГМЗ выше суммы остальных факторов почти в 1,5 раза. Наличие отягощенной по АГ наследственности среди персонала ГМЗ увеличивала вероятность АГ в 8,6-21,5 раза, СПЗ - в 5,4-15,7 раза. Наследственный фактор имел одинаковую роль в формировании АГ на обоих предприятиях, поскольку 95% CL для ОШ пересекаются (p>0,05).

Следующим по значимости фактором являлась СДО, роль которого среди рабочих ГМЗ была в 5,2 раза менее существенна в сравнении с наследственностью. При этом на каждый 1 мЗв у рабочих ГМЗ шанс наличия АГ увеличивался в 1,5 раза. Меньшее влияние на распространенность АГ оказывал показатель ИМТ: на каждую единицу избыточной массы тела (свыше 25 кг/м²) вероятность иметь АГ увеличивалась как на ГМЗ, так и на СПЗ в равной степени (в 1,05 раза).

Следующим по значимости фактором являлась ЛТ, которая повышала вероятность АГ в 0,54 раза (95% CL 0,48-0,87) у рабочих ГМЗ и в 0,21 раз (95% CL 0,09-0,45) у рабочих СПЗ на каждый балл шкалы тревожности, начиная с нижней границы уровня высокой ЛТ, причем различия

Таблица 3. Связи предикторов с АГ: бинарная логистическая регрессия

Предикторы АГ	ГМЗ			СПЗ		
	β-коэффициент	ОШ (95% CL)	p	β-коэффициент	ОШ (95% CL)	p
Наследственность (0=нет, 1=да)	2,609	13,58 (8,59-21,45)	<0,001	2,221	9,22 (5,43-15,66)	<0,001
СДО (мЗв)	0,514	1,51 (1,07-2,12)	<0,001	-	-	-
Продолжительность трудового стажа (лет)	-	-	-	0,15	1,01 (0,98-1,05)	0,01
ИМТ (кг/м ²)	0,053	1,05 (1,01-1,11)	0,028	0,003	1,0 (0,93-1,09)	0,03
ЛТ (баллы)	-0,381	0,54 (0,48-0,87)	0,01	-1,56	0,21 (0,09-0,45)	0,02
Употребление алкоголя (г/нед)	-0,693	0,51 (0,22-0,91)	0,001	-0,543	0,58 (0,19-0,96)	0,01

между предприятиями были статистически значимыми, что объясняется преобладанием среди рабочих ГМЗ лиц с высоким уровнем ЛТ.

Вклад употребления алкоголя как предиктора АГ на ГМЗ и СПЗ был сопоставим: ОШ составили 0,51 (95% CL 0,22-0,91) и 0,58 (95% CL 0,19-0,96), соответственно.

На распространенность АГ влияют множество взаимосвязанных факторов. Роль экспонирующего фактора в нашем исследовании - влияние факторов ураноопасного производства, главным образом ИИ, можно уточнить только при условии устранения влияния на распространенность АГ всех конфаундинг-факторов. Поэтому повышенный грубый RR АГ у персонала ГМЗ в сравнении с СПЗ не позволяет констатировать истинное повышение распространенности заболевания в данной группе. Более точное суждение о превышении распространенности того или иного заболевания возможно лишь на основании оценки стандартизованного относительного риска (SRR). Рассчитать SRR можно используя метод стратификации, который позволяет контролировать эффекты конфаундингов.

В нашем исследовании экспонирующим (изучаемым) фактором являлась принадлежность субъекта к ГМЗ, а конфаундингами - возраст и основные предикторы АГ: наследственность, ожирение, употребление алкоголя, уровень тревожности (ЛТ и РТ), злоупотребление солью, гиподинамия и СДО. Все перечисленные предикторы АГ можно разделить на бинарные (наследственность, пристрастие к соленой пище, низкая физическая активность, метаболический синдром), количественные непрерывные (ожирение - ИМТ, употребление алкоголя - г/нед) и номинальные порядковые (уровень тревожности). С целью упрощения процедуры стратификации все предикторы были приведены к бинарным.

Стратификация и расчёт SRR проводился с использованием программы Epi-info-2004.

В результате проведенного исследования рассчитан SRR АГ, который составил 2,91 (95% CL 2,1-3,8; $\chi^2=51,5$; $p<0,001$). Распространенность АГ у рабочих ГМЗ, даже при устранении всех конфаундингов, была в 2,9 раза выше, чем у персонала СПЗ.

Необходимо отметить, что SRR АГ был выше «грубого RR» (1,8), что свидетельствует о значимом влиянии СДО в сочетании с другими вредными факторами производства по переработке урановой руды на распространенность АГ. SRR измеряет силу связи между воздействием и заболеванием: чем сильнее влияние экспонируемого фактора, тем выше относительный риск. Поскольку мешающие факторы при подборе групп были исключены, имеются все основания считать, что повышенный SRR в экспонируемой группе является доказательством причинности производственных факторов в развитии заболевания.

Выводы. Распространенность АГ среди рабочих основных производственных цехов ГМЗ составила 23,9% (95% CL 21,0 – 26,8). RR АГ в экспонируемой по ИИ группе в сравнении с группой СПЗ был избыточным – 1,8 (95% CL 1,4 – 2,3). Психо-эмоциональное состояние рабочих ГМЗ характеризовалось повышенной личностной и реактивной тревожностью: RR высокой ЛТ и РТ были избыточными, составляя 21,6 (95% CL 13,8 – 33,8; $p<0,0001$) и 4,9 (95% CL 2,9 – 8,4; $p<0,0001$), соответственно. Распространенность остальных предикторов АГ - наследственность, ожирение, метаболический синдром, злоупотребление алкоголем, пристрастие к соленой пище, низкая физическая активность на сравниваемых предприятиях была сопоставима (RR \approx 1).

Наиболее весомыми факторами риска АГ у рабочих ГМЗ являласьотяоженная наследственность (ОШ 13,6; 95% CL 8,6 – 21,5; $p<0,0001$). Следующими по значимости факторами риска были СДО (ОШ 1,5; 95% CL 1,1 – 2,2; $p<0,0001$) и ИМТ (ОШ 1,1; 95% CL 1,0 – 1,1; $p=0,03$). Вклад употребления алкоголя и личностной тревожности как предикторов АГ был сопоставим (ОШ \approx 0,53). Рассчитанный нами SRR АГ ГМЗ vs СПЗ составил 2,91 (95% CL 2,1-3,8; $\chi^2=51,5$; $p<0,001$), т.е. риск болеть АГ у рабочих ГМЗ был в 2,9 раза выше, чем у рабочих СПЗ, при условии нивелирования основных предикторов заболевания.

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SUMMARY

ARTERIAL HYPERTENSION AMONG WORKERS OF A URANIUM PROCESSING ENTERPRISE OF THE REPUBLIC OF KAZAKHSTAN: PREVALENCE, RELATIVE RISKS AND PREDICTORS OF THE DEVELOPMENT

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The aim of this study was to establish the prevalence, relative risks, and predictive factors of the development of arterial hypertension (AH) in workers of a uranium processing enterprise (UPE).

An open cross-sectional comparative study of 809 UPE workers was conducted. The compared them to the 696 workers of the bearing plant, located at a distance of 10 km from UPE. Stratification was performed and odds ratios (OR), gross (RR) and standardized relative (SRR) risks of hypertension were calculated. Our results revealed a high prevalence of hypertension among UPE workers (24.8%), as well as greater risk of devel-

opment of hypertension among workers of UPE in comparison to the personnel of the non-uranium enterprise: RR=2.4 and SRR=2.9. The most significant predictors of hypertension were burdened heredity (OR = 13.6), total radiation dose (OR=1.5), overweight (OR=1.1), high anxiety (OR=0.5) and systematic use of alcohol (OR=0.5).

Thus, among workers chronically exposed to radiation toxicity, high prevalence of hypertension, excessive RR and SRR of developing hypertension and the presence of risk factors for hypertension were established.

Keywords: arterial hypertension, a uranium processing enterprise, prevalence, relative risks.

РЕЗЮМЕ

АРТЕРИАЛЬНАЯ ГИПЕРТЕНЗИЯ У РАБОЧИХ УРАНОПЕРЕРАБАТЫВАЮЩЕГО ПРЕДПРИЯТИЯ КАЗАХСТАНА: РАСПРОСТРАНЕННОСТЬ, ОТНОСИТЕЛЬНЫЕ РИСКИ И ПРЕДИКТОРЫ РАЗВИТИЯ

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Целью исследования явилось изучение распространенности, относительных рисков и предикторов развития артериальной гипертензии (АГ) у рабочих ураноперерабатывающего предприятия (УПП). Проведено открытое кросс-секционное сравнительное исследование 809 рабочих УПП. Группу сравнения составили 696 рабочих подшипникового завода, расположенного на расстоянии 10 км от УПП. Проведены стратификация и расчет отношения шансов (ОШ), грубых (RR) и стандартизованных относительных (SRR) рисков АГ. Результаты исследования выявили высокую распространенность (24,8%) АГ, а также большие риски развития АГ среди рабочих УПП в сравнении с персоналом неураноопасного производства: RR=2,4 и SRR=2,9. Наиболее весомыми предикторами АГ являлисьотяошенная по АГ наследственность (ОШ= 13,6), суммарная доза облучения (ОШ=1,5), избыточная масса тела (ОШ=1,1), высокий уровень тревожности (ОШ=0,5), систематическое употребление алкоголя (ОШ=0,5). Таким образом, среди рабочих, подвергавшихся длительному радиационно-токсическому воздействию, выявлены высокая распространенность АГ, избыточные RR и SRR и значимые предикторы АГ.

რეზიუმე

არტერიული ჰიპერტენზია ყაზახეთის ურანის გადამამუშავებელი საწარმოს მუშებში: გავრცელება, შედარებითი რისკები და განვითარების პრედიქტორები

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კვლევის მიზანს წარმოადგენდა არტერიული ჰიპერტენზიის გავრცელების შედარებითი რისკების და განვითარების პრედიქტორების შესწავლა ურან-

გადამამუშავებელი საწარმოს მუშებში. ჩატარდა ღია კროს-სექციური შედარებითი კვლევა 809 ურან-გადამამუშავებელი საწარმოს (უგს) მუშებში. საკონტროლო ჯგუფი შეადგინა გორგოლაჭების ქარხნის 696 მუშამ. ჩატარდა არტერიული ჰიპერტენზიის (აპ) შეფარდებითი ალბათობის (შა), უხეში და სტანდარტიზირებული რისკების (სრ) სტრატეფიკაცია და გაანგარიშება. კვლევის შედეგებმა გამოავლინა აპ-ის

გაერცვლების მაღალი მაჩვენებელი (24,8%) და მისი განვითარების მაღალი რისკები უგს მუშებს შორის გორგოლაჭების ქარხნის პერსონალთან შედარებით. აპ-ის მნიშვნელოვან პრედიქტორებს წარმოადგენს მემკვიდრეობითი ჰიპერტენზია (შა=13,6), დასხივების წამური დოზა (შა=1,5), სხეულის ჭარბი წონა (შა=1,1), შფოთვის მაღალი დონე და ალკოჰოლის მუდმივი მოხმარება (შა=0,5).

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