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ЕЖЕМЕСЯЧНЫЙ НАУЧНЫЙ ЖУРНАЛ

Медицинские новости Грузии

საქართველოს სამედიცინო სიახლენი

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გამოიცემა თბილისის სახელმწიფო სამედიცინო უნივერსიტეტთან
თანამშრომლობითა და მისი პატრონაჟით

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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GMN: Медицинские новости Грузии - ежемесячный рецензируемый научный журнал, издаётся Редакционной коллегией и Международной академией наук, образования, искусств и естествознания (IASEIA) США с 1994 года на русском и английском языках в целях поддержки медицинской науки и улучшения здравоохранения. В журнале публикуются оригинальные научные статьи в области медицины, биологии и фармации, статьи обзорного характера, научные сообщения, новости медицины и здравоохранения.

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GMN: Georgian Medical News – საქართველოს სამედიცინო სიახლენი – არის ყოველთვიური სამეცნიერო სამედიცინო რეცენზირებადი ჟურნალი, გამოიცემა 1994 წლიდან, წარმოადგენს სარედაქციო კოლეგიისა და აშშ-ის მეცნიერების, განათლების, ინდუსტრიის, ხელოვნებისა და ბუნებისმეტყველების საერთაშორისო აკადემიის ერთობლივ გამოცემას. GMN-ში რუსულ და ინგლისურ ენებზე ქვეყნდება ექსპერიმენტული, თეორიული და პრაქტიკული ხასიათის ორიგინალური სამეცნიერო სტატიები მედიცინის, ბიოლოგიისა და ფარმაციის სფეროში, მიმოხილვითი ხასიათის სტატიები.

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При представлении в печать научных экспериментальных работ авторы должны указывать вид и количество экспериментальных животных, применявшиеся методы обезболивания и усыпления (в ходе острых опытов).

4. К статье должны быть приложены краткое (на полстраницы) резюме на английском, русском и грузинском языках (включающее следующие разделы: цель исследования, материал и методы, результаты и заключение) и список ключевых слов (key words).

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2. Size of the article, including index and resume in English, Russian and Georgian languages must be at least 10 pages and not exceed the limit of 20 pages of typed or computer-printed text.

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.

5. Tables must be presented in an original typed or computer-printed form, instead of a photocopied version. **Numbers, totals, percentile data on the tables must coincide with those in the texts of the articles.** Tables and graphs must be headed.

6. Photographs are required to be contrasted and must be submitted with doubles. Please number each photograph with a pencil on its back, indicate author's name, title of the article (short version), and mark out its top and bottom parts. Drawings must be accurate, drafts and diagrams drawn in Indian ink (or black ink). Photocopies of the X-ray photographs must be presented in a positive image in **tiff format**.

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7. Please indicate last names, first and middle initials of the native authors, present names and initials of the foreign authors in the transcription of the original language, enclose in parenthesis corresponding number under which the author is listed in the reference materials.

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ავტორთა საყურადღებოდ!

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2. სტატიის მოცულობა არ უნდა შეადგენდეს 10 გვერდზე ნაკლებს და 20 გვერდზე მეტს ლიტერატურის სიის და რეზიუმეების (ინგლისურ, რუსულ და ქართულ ენებზე) ჩათვლით.

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6. ფოტოსურათები უნდა იყოს კონტრასტული; სურათები, ნახაზები, დიაგრამები - დასათაურებული, დანომრილი და სათანადო ადგილას ჩასმული. რენტგენოგრამების ფოტოასლები წარმოადგინეთ პოზიტიური გამოსახულებით **tiff** ფორმატში. მიკროფოტოსურათების წარწერებში საჭიროა მიუთითოთ ოკულარის ან ობიექტივის საშუალებით გადიდების ხარისხი, ანათალების შეღებვის 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 ფჩხილებში 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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НАУКА

MYELOLIPOMA COEXISTENCE WITH GLUCOCORTICOID AND ANDROGEN SECRETING ADRENOCORTICAL CARCINOMA: SLOW AND BENIGN CLINICAL COURSE

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Adrenal tumors are frequently detected in abdominal imaging. İn autopsy series, the prevalence of adrenal incidentalomas varies between 1-9%. However, the prevalence is higher in obese, diabetic and hypertensive patients [1]. Adrenocortical cancer(ACC) is very rare and has a poor prognosis. Five-year survival is approximately 50% in early stage disease and approximately 20% in advanced stage disease [2-5]. Adrenal myelolipomas (AML) contain varying amounts of hematopoietic elements and mature adipose tissue. It is a rare adrenal tumor and its incidence is 0.08–0.4% in autopsy series [6]. Adrenal myelolipomas are always nonfunctional, but they may occasionally be associated with congenital adrenal hyperplasia, cushing syndrome, conn syndrome and pheochromocytoma, which are functional disorders of the adrenal gland [7-9]. We herein report a relatively rare case of a myelolipoma with a adrenocortical cancer that grows slowly and becomes functional over time. In this report, we describe a case of adrenocortical cancer secreting glucocorticoid coexistent with a giant adrenal myelolipoma.

Case Presantion. A 48-year-old woman (body weight, 87 kg; height, 164 cm) presented at our outpatient clinic with a history of hypertension for about 5 years, and due to the development of acne lesions on the face. She said that she had wanted to become pregnant for the last 5 years, but the pregnancy has not occurred. There was no pregnancy history. She had a history of oligomenorrhea, weight gain, and heat intolerance for the last 1 year. She was in the perimenopausal period. Her past medical history was significant for having left giant adrenal tumor. Approximately 4 years ago, magnetic resonance imaging (MRI) revealed a mass of 110x70 mm in the left adrenal gland. At that time, cortisol suppression was found in the dexamethasone suppression test for functional evaluation. Due to mass size, removal of the tumor was recommended but she refused. No control evaluation was made within 4 years. Her father had diabetes and mother had hypertension. Patient's blood pressure and heart rate showed 150/90 mmHg and 78 beats/min respectively. She was taking amlodipine and olmesartan/hydrochlorothiazide for hypertension. There were acneic lesions on the face skin.

Laboratory findings. Complete blood count, renal function tests, thyroid function tests, liver function tests, and serum electrolytes were all within normal range (NR). Plasma renin activity, plasma aldosterone, twenty-four hours urine catecholamine and its metabolites were within the normal reference values. Cortisol levels after 1 mg and 2 mg dexamethasone suppression test were 11.3 mcg/dL and 10.6 mcg/dL, respectively. Basal serum ACTH level was <5 pg/mL. Although the ACTH level was suppressed, the DHEAS value was higher than the expected reference range for the patient's own age [DHEAS:409 mcg/dL(Reference range:56-282)]. Current laboratory findings indicated cushing syndrome and hyperandrogenism.

Radiological findings. Computed tomography revealed a heterogeneously contrasting mass of 145x118x100mm with lobular contour and soft tissue areas (Fig. 1). This mass was extending to the stomach and spleen at the top and compressing the left kidney at the bottom. Compared to the imaging report four years ago, there was an increase in mass size of approximately 30%.

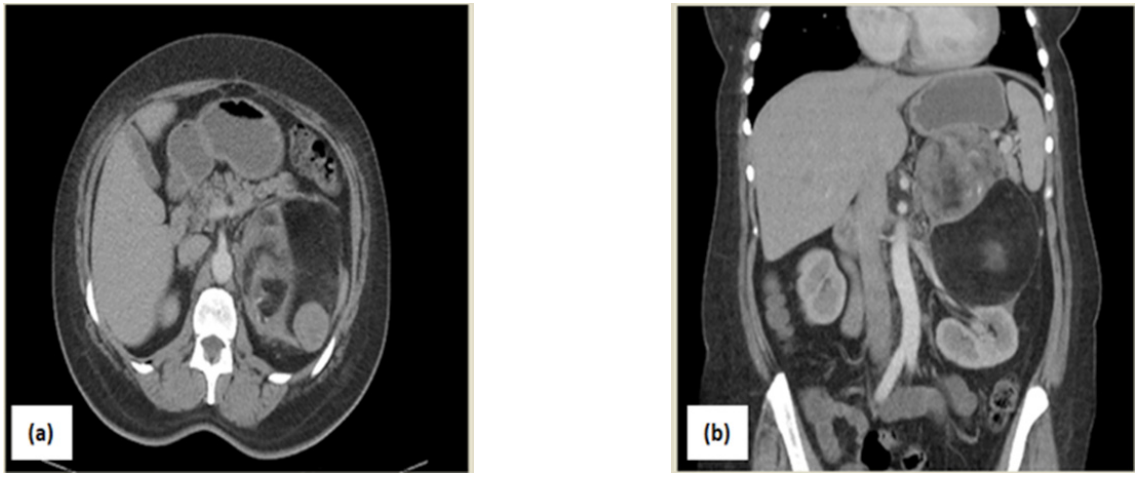

Fig. 1. Computed tomography (CT) scans. (a)CT shows a heterogeneous mass with lobular contour and soft tissue areas, (b)This mass was extending to the stomach and spleen at the top and compressing the left kidney at the bottom

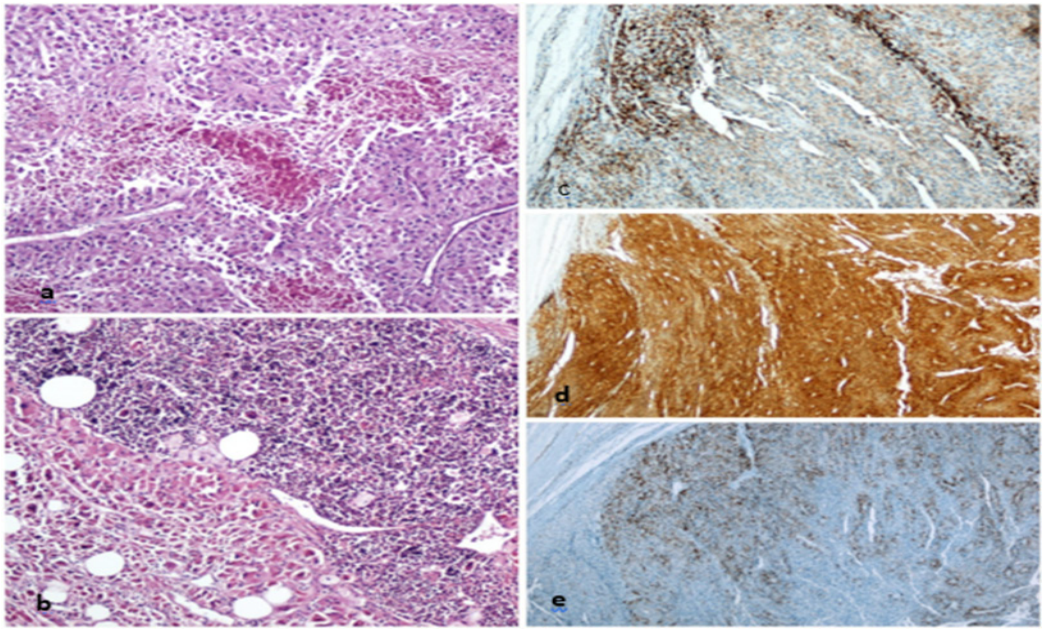

Fig. 2. Histologic section from tumor showing adrenocortical carcinoma and adrenal myelolipoma. (a) oncocytic type adrenocortical carcinoma with areas of necrosis (H&E X200), (b) adrenocortical cancer and adrenal myelolipoma foci showing in the same section (H&E X200), (c) Melan A positivity in tumor cells (Melan A X200), (d) positivity of diffuse synaptophysin (Synaptophysin X100), (e) focal inhibin positivity in tumor cells (Inhibin X100)

Surgical process. She was given surgery with steroid treatment according to Cushing protocol. Due to the compression effect of the mass, functional features, and atypical features of the mass, left adrenalectomy was deemed suitable. Laparoscopic transperitoneal left adrenalectomy was performed with three port technique. There were no complications in the perioperative period. The resected specimen weighed 850 grams. In the postoperative period, baseline cortisol 1.2mcg/dL, ACTH 24 pg/mL, DHEAS 8.4 mcg/dL were determined. Oral hydrocortisone treatment was continued at physiological dose because of steroid deficiency symptoms. Postoperative computed tomography showed no residual mass. Because of the presence of ACC, PET-CT was performed for possible metastasis. No metastasis was detected. Antihypertensive treatment was discontinued due to lack of need.

Pathological findings. Macroscopically, surgical material was 850 gr weighted and 15x14x10 cm seized. There was 3 surfaces encapsulated tumoral lesion, first and the largest one was 10 cm , the second was 4 cm adjacent to the first one , the third was 3 cm away and had 5 cm diameter. The cross-sectional face of the large tumor contains necrotic brown hemorrhagic areas. Circumference of the tumors was greasy. Samples were taken from the tumor and stained.

Microscopically; megakaryocytic, erythroid and lymphoid serial elements and in some areas bone lamellae and mature adipose tissue were observed in adrenal tissue. Also, oncocytic cells that have large hyperchromatic nuclei, eosinophilic nucleolus and nuclear pleomorphism and containing a small number of mitoses was seen in the tumoral tissue (Fig. 2).

Immunohistochemical analysis showed that the carcinoma cells were Synaptophysin, Calretinin diffuse positive, Inhibin, Melan A, and P53 focal positive, CD34, Chromogranin A, PanCK, HepPAR, S-100, CD-10 negative. Ki-67 proliferation index was 15%. The patient was diagnosed with myelolipoma and oncocytic variant adrenocortical cancer coexistence.

Despite the more frequent use of imaging techniques such as MRI and computed tomography, adrenal masses are still incidentally detected. Adrenal myelolipoma is a benign tumor composed of bone marrow-like hematopoietic elements and varying proportions of mature adipose. In imaging, a large amount of adipose tissue density is observed in myelolipomas [10]. These tumors are mostly detected between 5-7 decades and are usually detected equally in both sexes [11]. In addition, they are always nonfunctional and do not cause disorders in the hematopoietic system. Although hormone is inactive, these tumors may coexist with other diseases that endocrine dysfunction. For example, case reports of adrenal myelolipoma associated with congenital adrenal hyperplasia, 21-hydroxylase deficiency, 3-beta hydroxylase deficiency, primary hyperaldosteronism (conn syndrome), pheochromocytoma, and cushing syndrome have been reported [12-19]. In our case, we know that the adrenal mass was nonfunctional when it was first detected. In the following four years, we observed both an increase in mass size and autonomic glucocorticoid production.

Adrenocortical carcinomas are rare, accounting for about 1 case per 1 million in the population. They are highly aggressive tumors with a mortality rate of up to 50%. Approximately 60 percent of ACCs are secretory of hormone excess [20-24]. Adults with hormone-secreting ACCs often have only cushing syndrome. However, they occasionally have an overproduction of glucocorticoid and androgen, which causes a mixed Cushing's and virilization syndrome. ACC cases less than 10% can be presented only by virilization. However, if only virilization is present, the cause is more ACC than adenoma. In addition, muscle atrophy and skin weakness seen in cushing syndrome may not be observed if androgen excess is present in addition to glucocorticoid excess. In our case, although the ACTH was suppressed, DHEAS was found elevated for age and sex. Dexamethasone suppression tests were consistent with cushing syndrome. According to these

findings, both glucocorticoid and androgen levels were high. ACC associated with Cushing's syndrome leads to shorter survival due to the increased risk of infections and metabolic or vascular complications [25,26]. However, the clinical course was very slow in our patient and the surgical procedure was uneventful.

In the literature, fewer than 10 case reports have been published with ACC-myelolipoma coexistence [27-32]. In one case series, 49 ACCs were examined and 2 myelolipomas were identified but hormonal status was not specified [31]. Hyperaldosteronism was detected in one of the cases [27]. However, in other reports, cases were defined as nonfunctional [28,30]. Our case seems to be an unique in the literature in terms of hormonal status (both androgen and glucocorticoid hypersecretion).

In large adrenal tumors, excision is recommended because of the possibility of cortical cancer. In this report, the importance of this situation was clearly revealed. In summary, the giant adrenal tumors should be removed surgically even though having benign imaging characteristics. Also, the clinician should be alert to the non-functional adrenal tumor that begins to produce hormones over time.

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SUMMARY

MYELOLIPOMA COEXISTENCE WITH GLUCOCORTICOID AND ANDROGEN SECRETING ADRENOCORTICAL CARCINOMA: SLOW AND BENIGN CLINICAL COURSE

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We present a case of androgen and glucocorticoid secreting adrenocortical carcinoma with concomitant myelolipoma. A giant adrenal tumor which was initially nonfunctional was reassessed four years later due to the patient's refusal to treat. The patient was a 48-year-old woman with hypertension and acne lesions on the face. Laboratory findings were consistent with glucocorticoid and androgen hypersecretion. Computed tomography revealed a heterogeneously contrasting mass of 145x118x100 mm with lobular contour and soft tissue areas. The patient underwent left laparoscopic transperitoneal adrenalectomy with three port technique. There were no complications in the perioperative period. The resected specimen weighed 850 grams. Pathological findings showed a combination of myelolipoma-adrenal cortical cancer. In the postoperative period, hypertension improved and the hormone panel was normalized. Postoperative computed tomography and PET-CT showed no residual mass and metastasis. Although imaging is compatible with benign masses such as myelolipoma, coexistence of ACC-myelolipoma should be kept in mind and functional evaluation should be performed.

Keywords: Myelolipoma, Adrenocortical Carcinoma, Cushing Syndrome, Hyperandrogenism

РЕЗЮМЕ

СОЧЕТАНИЕ МИЕЛОЛИПОМЫ С ГЛЮКОКОРТИКОИДНОЙ И АНДРОГЕННОЙ СЕКРЕТИРУЮЩЕЙ КАРЦИНОМОЙ КОРЫ НАДПОЧЕЧНИКОВ: МЕДЛЕННОЕ И ДОБРОКАЧЕСТВЕННОЕ КЛИНИЧЕСКОЕ ТЕЧЕНИЕ

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Описан клинический случай андроген- и глюкокортикоид-секретирующей адренокортикальной карциномы коры надпочечников с сопутствующей миелолипомой. Гигантская атрофированная опухоль надпочечников, от лечения которой отказалась пациентка, была вновь осмотрена четыре года спустя. Лабораторные данные 48-летней пациентки с гипертонической болезнью и угревой сыпью на лице показали наличие гиперсекреции глюкокортикоидов и андрогенов. Компьютерная томография выявила неоднородно контрастную массу размером 145x118x100 мм с дольчатым контуром и участками мягких тканей. Пациентке выполнена левая лапароскопическая трансперитонеальная адреналэктомия по трехпортовой методике. Осложнений в периоперационном периоде не наблюдалось. Резецированный образец весил 850 гр. По результатам патологического исследования выявлено сочетание миелолипомы с раком коры надпочечников. В послеоперационном периоде улучшились показатели артериальной гипертензии, нормализовалась гормональная панель. Послеоперационная компьютерная томография и ПЭТ-КТ остаточной массы и метастазов не выявили. Визуализация показала соответствие опухоли таким доброкачественным образованиям, как миелолипома. Авторы не считают целесообразным исключение наличия адренокортикального рака и рекомендуют проведение функциональной оценки.

რეზიუმე

მიელოლიპომის თანაარსებობა თირკმელზედა ჯირკვლის ქერქის გლუკოკორტიკოიდულ და ანდროგენულ სეკრეციულ კარცინომასთან: ნელი და კეთილთვისებიანი კლინიკური მიმდინარეობა

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აღწერილია თირკმელზედა ჯირკვლის ქერქის გლუკოკორტიკოიდ- და ანდროგენ-სეკრეციული ადრენოკორტიკული კარცინომის კლინიკური შემთხვევა თანმხლები მიელოლიპომით. თირკმელზედა ჯირკვ-

ლის გიგანტური ატროფირებული სიმსივნე, რომლის მკურნალობაზეც პაციენტმა ქალმა უარი განაცხადა, განმეორებით განხილული იქნა ოთხი წლის შემდეგ. 48 წლის ასაკის პაციენტი ქალის მონაცემებმა,ჰიპერტონიული დაავადებით და აკნეთი სახეზე,აჩვენა გლუკოკორტიკოიდების და ანდროგენების ჰიპერსეკრეციის არსებობა. კომპიუტერული ტომოგრაფიით გამოვლინდა არაერთგვაროვანი მასა, ზომით 145x118x100მმ, წილოვანი კონტურებით და რბილი ქსოვილების მონაკვეთებით. პაციენტს ჩაუტარდა მარცხენამხრივი ლაპარასკოპიული ტრანსპერიტონეული ადრენალექტომია. პერიოპერაციულ პერიოდში გართულებები არ აღინიშნა. ამოკვეთილი ნიმუშის წონა იყო 850 გრ. პათომორფოლოგიური კვლევის შედეგების მიხედვით გამოვლინდა მიელოლიპომის თანაარსებობა თირკმელზედა ჯირკვლის ქერქის კიბოსთან. ოპერაციის შემდგომ პერიოდში არტერიული ჰიპოერტენზიის მაჩვენებლები გაუმჯობესდა, ნორმალიზდა ჰორმონული პანელი. ოპერაციის შემდგომი ტომოგრაფიით და პეტ-კომპიუტერული ტომოგრაფიით ნარჩენი მასა და მეტასტაზები არ გამოვლინდა. ვიზუალიზაციით დადგენილ იქნა სიმსივნის შესაბამისობა ისეთ კეთილთვისებიან წარმონაქმნთან, როგორიცაა მიელოლიპომა. ავტორები დაასკვნიან, რომ ადრენოკორტიკული კიბოს გამორიცხვა არ შეიძლება და რეკომენდებულად თვლიან ფუნქციური შეფასების განხორციელებას.

ИЗОЛИРОВАННАЯ ПРОФУНДОПЛАСТИКА (ДИФФЕРЕНЦИРОВАННЫЙ ВЫБОР)

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При атеросклеротическом поражении артерий бедренно-подколенно-берцового сегмента глубокая артерия бедра (ГАБ) может длительно оставаться интактной, а ее многочисленные коллатеральные анастомозы способны компенсировать кровоток в голени и стопе. При данном сегменте поражения к основным коллатералям можно отнести нисходящую ветвь латеральной огибающей артерии бедра и прободающие артерии глубокой артерии бедра, которые анастомозируют с верхними и нижними коленными, икроножными артериями, передней и задней поворотными большеберцовыми артериями. При стенозе устья ГАБ, окклюзионно-стенотическом поражении ветвей проксимальной части подколенной, окклюзии подколенной и магистральных артерий голени наступает декомпенсация коллатерального кровотока [5,6,8,10-14].

Профундопластика объединяет хирургические вмешательства, которые восстанавливают просвет начального отдела ГАБ. В зависимости от типа пластичного материала выделяют два вида изолированной профундопластики: аутовенозную, аутоартериальную и с использованием аллопластического материала [1,4,9,12].

Однако у хирургов нет единого мнения относительно показаний к подобным операциям, отсутствует четкий алгоритм действий при подобных вмешательствах, нет регламентирующих критериев относительно выбора способа профундопластики, недостаточно глубоко изучены варианты хирургической анатомии ГАБ. Вышеизложенное диктует необходимость продолжить научные разработки по вопросу хирургических способов лечения хронической ишемии нижних конечностей, когда прямые и эндоваскулярные методы лечения не показаны конкретному пациенту.

Цель исследования - улучшить результаты лечения больных хронической ишемией нижних конечностей на основе оптимизации техники операций на глубокой артерии бедра.

Материал и методы. В течение 6 лет (2014-2019 гг.) в отделении сосудистой хирургии Закарпатской областной клинической больницы им. Андрея Новака, клинической базы ГВУЗ «Ужгородский национальный университет» первично прооперировано 150 больных по поводу облитерирующего атеросклероза бедренно-подколенно-берцового сегмента нижних конечностей. В демографической структуре пациентов значительно преобладали мужчины (90%, p<0,00001). Средний возраст больных составил 61,4±8,7 лет. При этом средний возраст женщин (65,6±7,9) почти на 5 лет превышал средний возраст мужчин (60,9±8,6) на время операции (t=5,77, p<0,00001). Все 150 больных имели окклюзию поверхностной артерии бедра с окклюзионно-стенотическим поражением подколенной артерии и артерий голени.

Ишемия нижних конечностей II степени отмечалась у 11 (7,3%) пациентов, III-А степени - у 63 (42%), III-Б степени - у 55 (36,7%) и IV степени - у 21 (14%). Среди сопутствующих заболеваний преобладали ишемическая болезнь сердца (77,3%), артериальная гипертензия (76,7%), эрозивные и эрозивно-язвенные поражения ЖКТ (52,7%), сахарный диабет (36,7%), хронические обструктивные заболевания легких (32,7%), последствия острых нарушений мозгового кровообращения (20,7%).

Все больные в зависимости от степени распространения окклюзионного поражения ГАБ были разделены на три группы: I группа - с преимущественным поражением устья ГАБ - 99 (66%) пациентов;

II группа - с поражением ГАБ от устья до второй латеральной прободающей артерии - 35 (23,3%) больных;

III группа - с поражением ГАБ до третьей латеральной прободающей артерии - 16 (10,7%) пациентов.

При этом редукция основного ствола у 40% больных составила 60%, у 45% пациентов - от 60 до 90%, у 15% - редукция диаметра оказалась более 90%.

В большинстве случаев для постановки диагноза достаточно сбора анамнеза и физикального обследования; для его верификации - определение лодыжечно-плечевого индекса (ЛПИ), измерение сегментарного давления в конечностях, определение глубокобедренно-подколенного индекса (ГБПИ), ультразвуковое дуплексное сканирование (УЗДС) и/или ультразвуковая допплерография (УЗДГ). Среди дополнительных инструментальных методов исследования использовали рентгенконтрастную ангиографию (РКА), мультиспиральную компьютерную томографию (МСКТ), измерение транскутанного напряжения кислорода (tcp02) в бассейне задней большеберцовой, передней большеберцовой, малоберцовой и артерий стопы перед операцией и в отдаленном послеоперационном периоде.

Для определения функциональной возможности реваскуляризации глубокой бедренной артерии определяли ГБПИ по формуле:

$$\text{ГБПИ}=\frac{\text{ВК}-\text{НК}}{\text{ВК}},$$

где ВК - регионарное систолическое давление в ПА выше колена,

НК - регионарное давление в ПА ниже колена.

Для изучения корреляции продолжительности сохранения конечности с ГБПИ осуществляли построение модели Кокса, которая выражает функцию риска следующим образом:

$$h(t)=h_0(t)\times\exp(\beta x),$$

где $h_0(t)$ - функция базового риска, h(t) - функция риска, x - значение ковариаты, β - регрессионный коэффициент, экспонента которого является соотношением рисков при изменении значения ковариаты на единицу.

Модель Кокса является моделью пропорциональных рисков: не налагая никаких ограничений на вид функции базового риска, модель прогнозирует, что соотношение рисков вследствие различия в значении ковариаты не зависит от времени (коэффициент β не зависит от времени t).

Всем больным выполняли изолированную профундопластику (таблица 1). Как видно из таблицы 1, 71,2% больным выполнялась эндартерэктомия с аутовенозной и/или аутоартериальной пластикой. Операции глубокобеденного протезирования выполняли у 20% пациентов, шунтирование - у 6%. Реимплантация прободающих и огибающих ветвей выполнена у 20% больных.

Критериями хороших результатов после проведения изолированной профундопластики считали снижение уровня периферической гипоксии тканей конечности (дистанционное увеличения ходьбы до 500 м, заживление некротических ран), повышение объемной скорости кровотока (ОСК) в нижних конечностях, повышение уровня ЛПИ больше чем на 50% от стартовых показателей.

Удовлетворительным результатом считали уменьшение ишемии тканей (увеличение дистанции ходьбы до 200-300 м), исчезновение болевого синдрома в покое, наличие тенденции к заживлению ран и повышение показателей ОСК и ЛПИ на 30-50% по отношению к стартовым показателям.

Результаты считали неудовлетворительными, если после операции симптомы ишемии имели тенденцию к росту либо не исчезали, а также показатели регионарной гемодинамики не повышались более 10% от стартовых. Такие больные, в подавляющем большинстве, требовали выполнения высокой ампутации на уровне бедра в отдаленном послеоперационном периоде.

Результаты и их обсуждение. При сравнении радиологических методов диагностики артерий бедренно-подколенно-берцового сегмента и глубокой артерии бедра наиболее эффективным оказались МСКТ (89,1% и 90%, соответственно) и ультразвуковое дуплексное сканирование (88,7% и 82%, соответственно).

МСКТ, ангиографический и допплеровский анализ позволил выделить следующие типы поражения артерий дистального русла:

Таблица 1. Способы и количество выполненных профундопластик

Вид вмешательства	Количество	%
Изолированная профундопластика с аутовенозной заплатой	64	42,6
Протяженная профундопластика с аутовенозной заплатой	26	17,3
Открытая эндартерэктомия с алозаплатой	1	0,7
Открытая эндартерэктомия с аутоартериальной заплатой:	17	11,3
- по Weibel	7	4,7
- по Bertolucchi	7	4,7
- по Feldhaus	3	2
Приустьевая резекция ГАБ с аутовенозным протезированием	3	2
Бедренно-глубокобедренное алопротезирование:	7	4,7
- бедренно-глубокобедренное алопротезирование	6	4
-бедренно-глубокобедренное композитное алопротезирование	1	0,7
- с реимплантацией прободающих ветвей	5	3,3
Бедренно-глубокобедренное аутовенозное шунтирование	9	6
Бедренно-глубокобедренное аутовенозное протезирование	23	15,3
- с имплантацией прободающих ветвей	15	10
- с имплантацией огибающих ветвей	3	2
Всего	150	100

- окклюзионно-стенотические поражения ПА, артерии голени проходимые;

- окклюзионно-стенотические поражения ПА, окклюзия одной-двух артерий голени;

- окклюзионно-стенотические поражения ПА, диффузное окклюзионно-стенотическое поражение трех артерий голени;

- диффузное поражение артерий бедренно-подколенно-берцового сегмента с или без проходимой одной из артерий голени.

Показатель ЛПИ напрямую зависел от уровня и распространенности атеросклеротического поражения конечностей, степени ишемии и варьировал в пределах 0,56±0,12-0,24±0,02.

При локальных стенозах ГАБ преимущественно наблюдали гомогенные бляшки, у 82,7% наблюдали гетерогенные бляшки, содержащие смешанную структуру и чаще встречающиеся при распространенных стенозах.

У пациентов с ишемией II-Б степени показатель ГБПИ находился в пределах 0,27-0,44, с III-А степени 0,3-0,57, с III-Б степени - 0,34-0,58, с IV степени - 0,37-0,60.

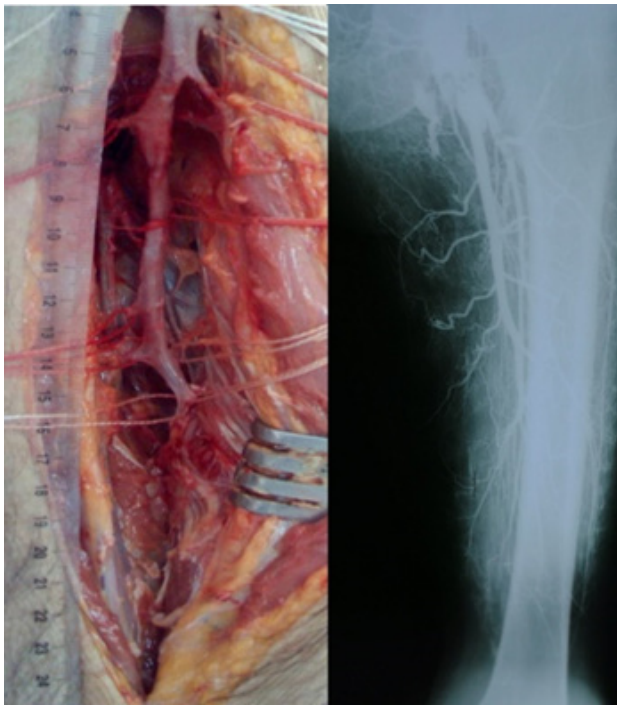
Известно, что анатомия ГАБ вариабельна. Сделать конечный вывод об истинной картине бассейна ГАБ возможно только при интраоперационной ревизии (рис. 1).

На основе данных интраоперационной ревизии выделено несколько вариантов формирования и отхождения ГАБ от общей артерии бедра (ОБА), а также расположение ее устья по отношению к ОБА:

- при первом варианте есть выраженный основной ствол ГАБ, от которого четко отходит латеральная и медиальная огибающие ветви в разной последовательности;

- при втором варианте строения латеральная и медиальная огибающие ветви и ГАБ отходят отдельно от ОБА;

- при третьем варианте характерно наличие только двух стволов, один из них - латеральная огибающая ветвь, второй - ГАБ.



а *б*

Рис. 1. Отпрепарированная ГАБ на протяжении 17 см (а). Рентгеноконтрастная ангиография: контрастированная ГАБ с 5-ю прободающими ветвями (б)

Учитывая мировой и собственный опыт при хирургическом лечении атеросклеротических окклюзионно-стенотических поражений глубокой артерии бедра следует заметить, что при наличии «коротких» стенозов предпочтение следует отдавать открытым эндартерэктомиям (ЕАЕ) с аутовенозной и/или аутоартериальной заплатой. В случае наличия у пациента выраженного кальциноза на конкретном протяжении, резекцию этого участка целесообразнее выполнить с аутовенозной и/или аутоартериальной пластикой. Даже незначительное сужение просвета ГАБ при окклюзии бедренной артерии сопровождается ухудшением коллатерального кровотока [1,4-6].

Таким образом, восстановление и/или расширение просвета происходит только за счет эндартерэктомии и заплат, в основном, это известные классические варианты: модификация Martina, Weibel, Bertolucchi, Feldhaus, бедренно-глубокобедренное протезирование (рис. 2-7).

Рис. 2-7. Классические варианты профундопластики.

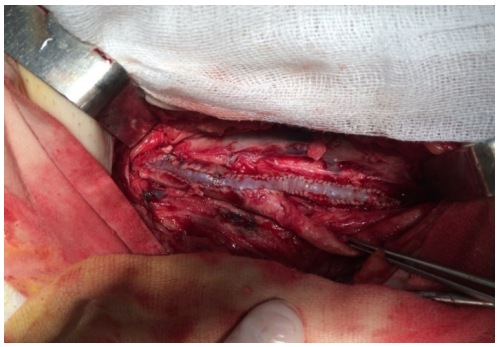


Рис. 2. Интраоперационное фото пациента С. Протяжная профундопластика с аутовенозной заплатой

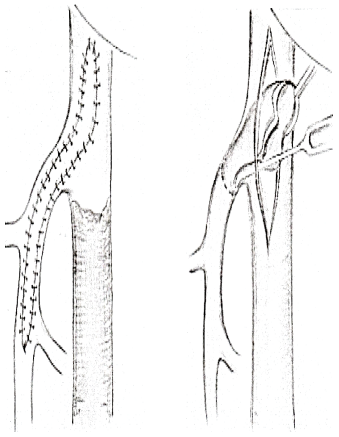


Рис. 3. Профундопластика в модификации Martin

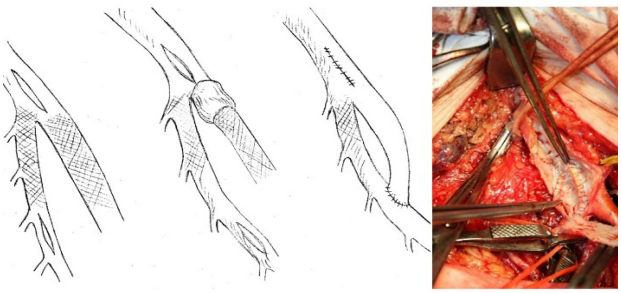


Рис. 4. Профундопластика в модификации Feldhaus

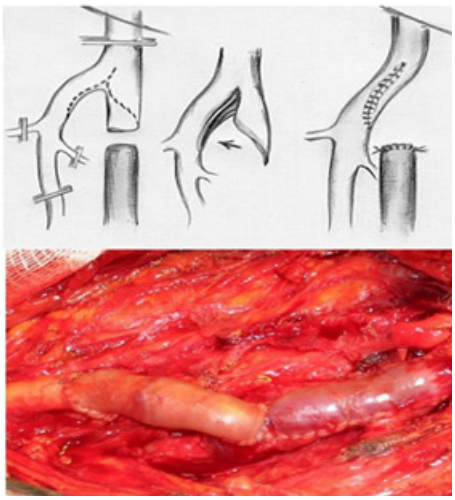


Рис. 5. Профундопластика в модификации Weibel с применением клювовидной аутоартериальной вставки

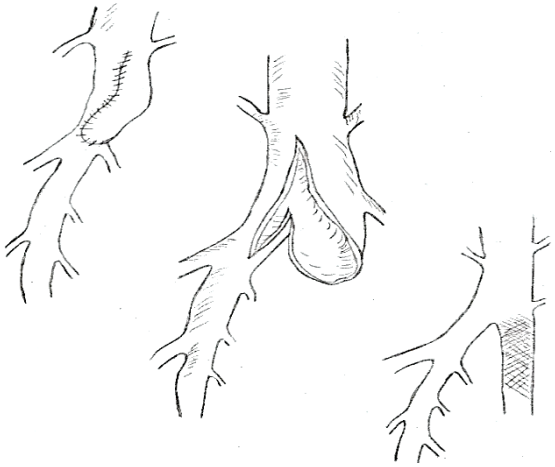


Рис. 6. Профундопластика в модификации Bertolucchi

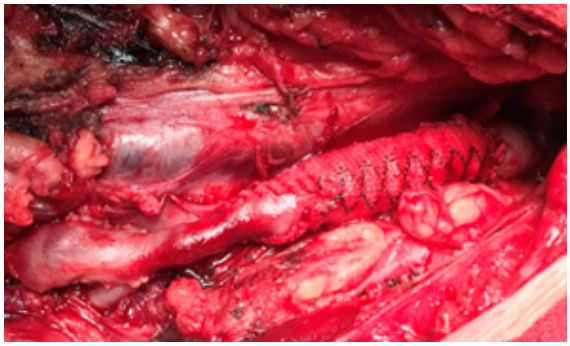


*Рис. 7. Интраоперационное фото пациента С.
Комбинированное бедренно-глубокобедренное протезирование (протез + вена)*

Таблица 2. Критерии выбора метода профундопластики в зависимости от протяженности, сегмента поражения и плотности атеросклеротической бляшки

Протяженность стеноза	**Степень поражения**	**Структура бляшки**	**Вид реконструкции**
До 4 см	Стеноз ГАБ >70% (окклюзия)	«+» «+-» «-»	Открытая ЕАЕ с аутовенозной или алозаплатой. Открытая ЕАЕ с аутоартериальной заплатой. Открытая ЕАЕ с аутовенозной заплатой.
4-10 см	Стеноз ГАБ >70% (окклюзия)	«+» «+-» «-»	Профундопластика аутоартериальной вставкой. Открытая ЕАЕ с аутовенозной заплатой. Бедренно-глубокобедренное протезирование с реимплантацией боковых ветвей
>10 см	Стеноз ГАБ >70% (окклюзия)	«+», «+-» «-»	Бедренно-глубокобедренное аутовенозное протезирование или шунтирование. Открытая ЕАЕ с аутовенозной заплатой
Диффузное поражение	Протяжный стеноз с окклюзией	«+» «+-» «-»	Профундопластика не показана

*примечание: «+» - плотная атеросклеротическая бляшка;
«+ -» - атеросклеротическая бляшка средней плотности; «-» - мягкая атеросклеротическая бляшка*

При протяженных стенозах суть профундопластики опять сводится к восстановлению и расширению просвета путем эндартерэктомии с аутовенозной заплатой и/или за счет шунтирования или протезирования сегмента ГАБ. Однако на участке ГАБ, который подлежит реконструкции, отходят боковые ветви, огибающие и прободающие ветви, представляющие интерес в плане восстановления в них кровотока, на что впервые обратил внимание Штутин А.А. [9]

Основной вопрос - это шунтирование или протезирование ГАБ при протяженных стенозах и почему именно так?

На это непосредственно влияет плотность атеросклеротической бляшки, которая определяет адекватность открытой эндартерэктомии. Чем тверже бляшка, чем сильнее поражения tunica media, тем больше снижается качество дезоблитерации и в таких случаях лучше выполнить протезирование (таблица 2).

На выбор реконструкции также влияет наличие огибающих и прободающих ветвей на месте необходимого уровня дезоблитерации. Если диаметр последних превышает 2 мм и по ним сохранен ретроградный кровоток, то лучше выполнить протезирование этого участка с реимплантацией боковых ветвей. Если боковых ветвей на протяжении участка поражения нет, показано бедренно-глубокобедренное шунтирование (рис. 8-13).

Таким образом, при сохраненном просвете и интактной стенке боковых ветвей ГАБ показана их реимплантация (рис. 8). Для упрощения выполнения реимплантации ветку выкраивают на площадке по окружности вокруг устья. При необходимости выполняют эверсионную эндартерэктомию из устья, после чего ветку вшивают по типу «конец-в-бок».

При плотно расположенных рядом боковых ветвях на пораженных атеросклерозом участках формируется косой «длинный» анастомоз на проксимальной части ГАБ после эндартерэктомии, из устья ветвей тем самым избегается реимплантация указанных ветвей в протез. Дистальный анастомоз формируется по типу «конец-в-конец» (рис. 9).

При варианте отхождения латеральной и медиальной огибающих ветвей от ОБА, и/или при наличии только двух стволов, один из них - латеральная огибающая ветвь, а второй - ГАБ, возможно выполнение протезирования пораженного участка с реимплантацией в трансплантат всех ветвей, стволов. Подобную тактику можно использовать при окклюзии ОБА и/или ее стенозе, который технически не поддается реверсийной ЕАЕ. Таким образом, выполняется бедренно-глубокобедренное протезирование с реимплантацией боковых ветвей (рис. 10).

Если не удается выполнить полноценную эндартерэктомию ОБА с приустьевым освобождением атероматоза, в таких случаях дистальнее уровня поражения ствола, ГАБ отсекается и имплантируется в близь расположенную прободающую ветвь (рис. 11).

В отдельных случаях возможно применить технику Feldhaus дезоблитерации просвета поверхностной артерии бедра (ПАБ) и использовать ее в качестве аутоартериального трансплантата для протезирования окклюзированной ветви ствола ГАБ путем артериальной профундопластики дезоблитерированной культи ПАБ в ГАБ по типу «конец-в-бок» (рис. 12).

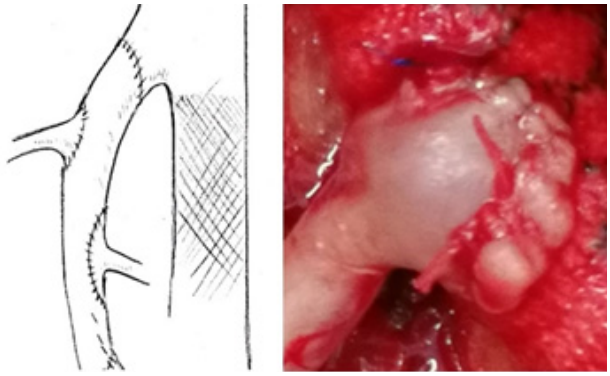


Рис. 8. Бедренно-глубокобедренное аутовенозное протезирование с имплантацией огибающих ветвей

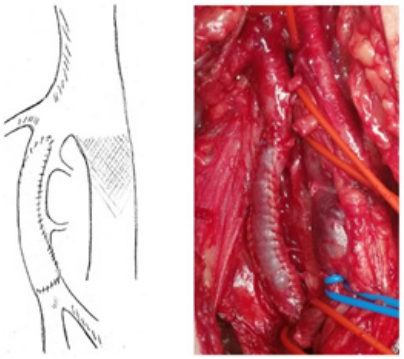


Рис. 9. Протезирование ствола ГАБ с формированием «косого» анастомоза «конец-в-конец»

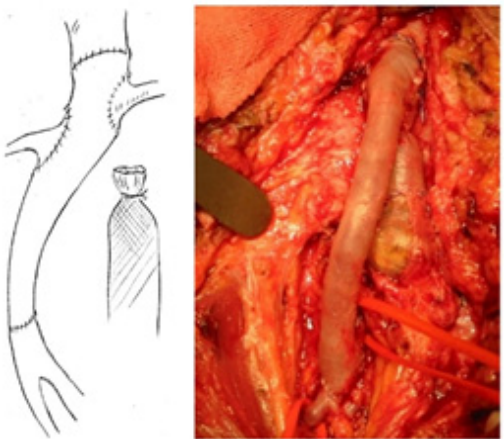


Рис. 10. Аутовенозное бедренно-глубокобедренное протезирование с реимплантацией медиальной, латеральной огибающей и прободающей ветвей в аутовенозный протез

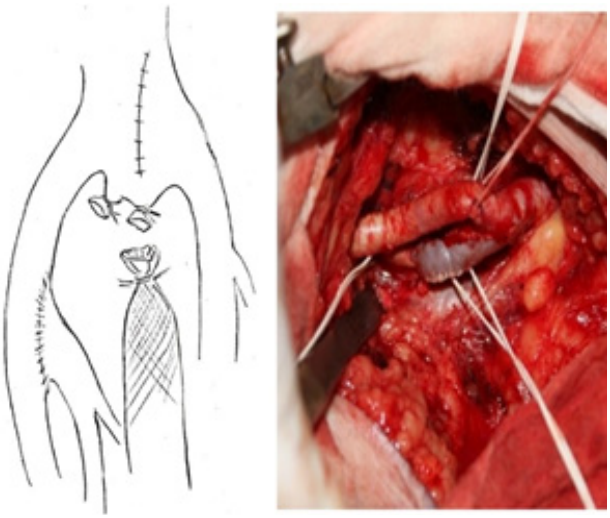


Рис. 11. Реимплантация ГАБ дистальнее уровня поражения ствола в близь расположенную прободающую ветвь

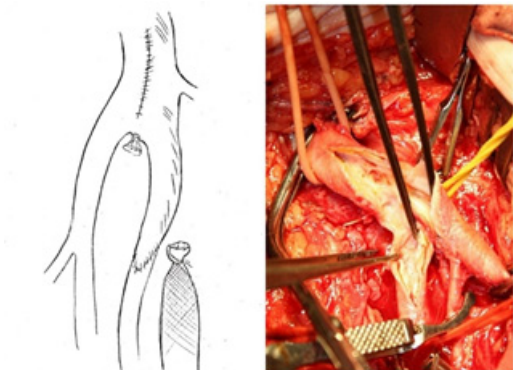


Рис. 12. Этап подготовки к аутоартериальной профундопластике ГАБ по типу «конец-в-бок»

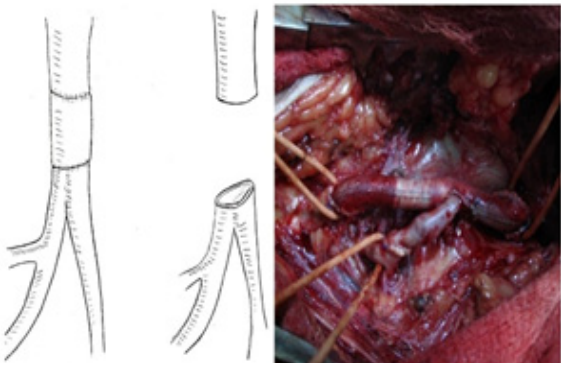


Рис. 13. Аутовенозное приустьевое протезирование общей бедренной артерии

При плотных атеросклеротических бляшках, стенозирующих просвет общей бедренной артерии до полной окклюзии, прикрывая при этом устье ГАБ, возможна приустьевая резекция сужения ГАБ с интерпозицией венозного трансплантанта БПВ сшитыми друг с другом в продольном направлении для увеличения диаметра аутовенозного протеза (рис. 13).

Ближайшие результаты профундопластики у пациентов с атеросклеротическим поражением бедренно-тибиального сегмента оценивались в течение 1 месяца после операции на основании изменения дооперационно клинической симптоматики, а также наличия или отсутствия различных послеоперационных осложнений. Так, на госпитальном периоде успех реваскуляризации, о чем свидетельствует проходимость зоны реконструкции, составил 100%. Летальных случаев ни в одной группе не отмечено.

Среди местных послеоперационных осложнений раннего послеоперационного периода наиболее часто отмечалась лимфорея, которая наблюдалась у 8 (5,3%) больных и подвергалась консервативной терапии во всех случаях. Нагноение послеоперационной раны наблюдалось у 3 (2%) больных, которые вылечены консервативно.

Тромбозы зон реконструкции наблюдали у одного (0,7%) больного. Выполненная тромбэктомия показала положительный эффект.

На количество положительных результатов выполненных операций в отдаленном периоде незначительное влияние оказывала начальная степень ишемии конечности. При ишемии II-Б - III-А степени непосредственные положительные результаты получены в 97,3% случаях, в группе пациентов с III-Б ишемией количество положительных результатов составило только 74,5%. У больных с IV степенью ишемии и поражением всех берцовых артерий положительные результаты изолированной профундопластики наблюдались только у 61,9% больных.

Количество положительных результатов изолированных профундопластик составило 73,9%. Операции не показали клинического улучшения в 18,7% случаев, из них 7,4% больных не отмечали клинического улучшения, несмотря на увеличение лодыжечно-плечевого индекса (ЛПИ) на 0,2. Ампутации в данной группе больных выполнены в 3,3% случаев. В 18,7% на всю группу неудовлетворительных результатов проведенных операций выполнено 7 (4,7%) ампутаций.

При сравнении проходимости зоны реконструкции спустя 5 лет достоверных различий не было у пациентов с изолированной профундопластикой (86,9%) и протяжной профундопластикой (88,5%) не выявлено ($p<0,05$). ЛПИ спустя 5 лет был выше у пациентов с протяжной профундопластикой (0,65±0,07) в сравнении с изолированной профундопластикой (0,58±0,09; $p<0,05$). Данный феномен, по всей вероятности, связан с более объемным кровотоком, который осуществляется за счет эластичных возможностей пластического аутовенозного материала для протяжной профундопластики в сравнении с ригидной атеросклеротически измененной стенкой аутоартериальной заплаты.

Индекс региональной перфузии (ИРП) $tcpO_2$ до и после профундопластики характеризовался увеличением напряжения O_2 в бассейне передне большеберцовой артерии (ПББА) на 55,5%, задне большеберцовой артерии (ЗББА) на 35,3%, МБА на 33,3%, коже тыла стопы на 66,7%, коже подошвы на 44 4% (таблица 3, рис. 14).

Для прогнозирования эффективности вмешательств на глубокой артерии бедра предложена математическая модель, построенная на сравнении величины ГБПИ перед простым использованием вида операции, где при значении индекса ≤0,36 5-летнее сохранение конечности при профундопластике достигает 62%, а при значении индекса ≤0,35 - 74,2% сохранение конечности, при значении индекса 0,3 - 87,3%.

Очевидно, это связано с потенциально большими функциональными возможностями глубокобедренно-подколенной коллатеральной системы для полноценной реваскуляризации конечности, а также «гемодинамической адекватностью» периферического русла при выполнении профундопластики [2].

Все случаи ампутации имели место при величине ГБПИ >0,37. При этом в группе больных с ампутацией средняя величина ГБПИ составила 0,37±0,03 ($p<0,05$).

Таблица 3. Индекс региональной перфузии $tcpO_2$ до и после профундопластики

	ПББА	ЗББА	МБА	Тыл стопы	Подошва
Норма (n=31)	1,5 ±0,14	1,85±0,20	1,7±0,15	1,4±0,16	1,1±0,114
До операции (n=19)	0,5±0,05	0,61±0,01	0,4±0,03	0,4±0,04	0,4±0,05
После операции (n=19)	0,9±0,04	1,7±0,25	1,2±0,11	0,6±0,02	0,9±0.28

$p<0,05$

Таким образом, нами предпринята попытка выделить основные условия, которые определяют возможности использования ГАБ как основного коллатерального сосуда, который способен обеспечить кровоснабжение нижней конечности при ее атеросклеротическом поражении:

- наличие локальной окклюзии устья или стеноза глубокой бедренной артерии >70%;
- окклюзия поверхностной бедренной артерии, диффузное окклюзионно-стенотическое поражение подколенной артерии и артерий голени;
- ГБПИ в пределах <0,37;
- ЛПИ <0,45.

Выводы.

1. При протяженности стеноза ГАБ до 4 см независимо от плотности бляшки рекомендуется открыта ЕАЕ с аутовенозной, аутоартериальной и/или аловставкой.
2. При протяженности стеноза от 4 до 10 см с мягкой или средней плотности атеросклеротической бляшкой показана открытая ЕАЕ с аутовенозной заплатой. При плотных атеросклеротических бляшках предпочтение следует отдавать бедренно-глубокобедренному аутовенозному шунтированию или протезированию. При протяженности поражения более 10 см независимо от плотности атеросклеротической бляшки рекомендуется бедренно-глубокобедренное шунтирование и/или протезирование.
3. При протезировании ГАБ и диаметре прободающих и/или огибающих артерий ≥2 мм и сохраненном ретроградном кровотоке показана их реимплантация на площадке в протез.

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SUMMARY

ISOLATED PROFUNDOPLASTY (DIFFERENTIAL CHOICE)

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Objective - to improve the results of treatment of patients with chronic ischemia of the lower extremities based on optimization of the technique of operations on the deep femoral artery. During 6 years (from 2014 to the end of 2019), 150 patients were initially operated on for obliterating atherosclerosis of the femoropopliteal-tibial segment of the lower extremities in the department of vascular surgery of the Andrey Novak Regional Clinical Hospital, the clinical base of the Uzhgorod National University.

In the presence of "short" stenoses, preference should be given to open endarterectomy with an autovenous and / or autoarterial patch. With extended stenoses, the essence of profundoplasty is again reduced to the restoration and expansion of the lumen by endarterectomy with an autovenous patch and/or by bypassing or prosthetics of the DFA segment. The choice of reconstruction is also influenced by the presence of enveloping and piercing branches at the site of the required level of disinfection.

If the length of the DFA stenosis is up to 4 cm, regardless of the plaque density, it is recommended to open the EAE with autovenous, autoarterial and/or aloinsertion. With a stenosis length of 4 to 10 sm with a soft or medium density atherosclerotic plaque, an open EAE with an autovenous patch is

shown. If the lesion is more than 10 cm long, regardless of the density of the atherosclerotic plaque, femoral-deep femoral shunting and/or prosthetics are recommended. When prosthetics of the DFA and the diameter of the perforating and/or circumflex arteries is ≥2 mm and the preserved retrograde blood flow, their reimplantation on the site into the prosthesis is indicated.

Keywords: deep femoral artery, ischemia of the lower extremities, obliteration atherosclerosis of the lower extremities vessels, profundoplasty, transcutaneous oxygen tension.

РЕЗЮМЕ

ИЗОЛИРОВАННАЯ ПРОФУНДОПЛАСТИКА (ДИФФЕРЕНЦИРОВАННЫЙ ВЫБОР)

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Цель исследования - улучшить результаты лечения больных хронической ишемией нижних конечностей посредством оптимизации техники операций на глубокой артерии бедра.

В течение 6 лет (2014-2019 гг.) в отделении сосудистой хирургии Закарпатской областной клинической больницы им. Андрея Новака, клинической базы ГВУЗ «Ужгородский национальный университет» первично прооперировано 150 больных по поводу облитерирующего атеросклероза бедренно-подколенно-берцового сегмента нижних конечностей. Средний возраст больных составил 61,4±8,7 лет.

При наличии «коротких» стенозов предпочтение следует отдавать открытым эндартерэктомиям (ЕАЕ) с аутовенозной и/или аутоартериальной заплатой. При протяженных стенозах суть профундопластики сводится к восстановлению и расширению просвета путем ЕАЕ с аутовенозной заплатой и/или за счет шунтирования или протезирования сегмента глубокой артерии бедра (ГАБ). На выбор реконструкции влияет наличие огибающих и прободающих ветвей на месте необходимого уровня дезоблитерации.

При протяженности стеноза ГАБ до 4 см независимо от плотности бляшки рекомендуется открытая ЕАЕ с аутовенозной, аутоартериальной и/или аловставкой. При протяженности стеноза от 4 до 10 см с мягкой или средней плотности атеросклеротической бляшкой показана открытая ЕАЕ с аутовенозной заплатой. При протяженности поражения более 10 см независимо от плотности атеросклеротической бляшки рекомендуется бедренно-глубокобедренное шунтирование и/или протезирование. При протезировании ГАБ и диаметре прободающих и/или огибающих артерий ≥2 мм и сохраненном ретроградном кровотоке показана их реимплантация на площадке в протез.

რეზიუმე

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კვლევის მიზანს წარმოადგენდა ქვედა კიდურების ქრონიკული იშემიის მქონე პაციენტების მკურნალობის შედეგების გაუმჯობესება ბარძაყის ღრმა არტერიაზე ოპერაციის ტექნიკის გაუმჯობესების საშუალებით.

6 წლის განმავლობაში (2014 – 2019 წწ.) ა. ნოვაკის სახ. ზაკარპატიეს კლინიკური საოლქო საავადმყოფოს სისხლძარღვთა ქირურგიის განყოფილებაში პირველადად ნაოპერაციებია 150 პაციენტი ქვედა კიდურების ბარძაყ-მუხლქვეშა-წვივის სეგმენტის მაობლიტირებელი ათეროსკლეროზის გამო; პაციენტების საშუალო ასაკი - 61,4±8,7 წელი.

"მოკლე" სტენოზების არსებობის შემთხვევაში უპირატესობა უნდა მიეცეს ღია ენდარტერექტომიას აუტოვენური და/ან აუტოარტერიული ნაკერით. გახანგრძლივებული სტენოზების დროს პროფუნდოპლასტიკის არსი მდგომარეობს სანათურის აღდგენასა და გაფართოებაში ენდარტერექტომიით აუტოვენური ჩანართის გამოყენებით და/ან შუნტირებით, ან ბარძაყის ღრმა არტერიის პროთეზირებით. რეკონსტრუქციის არჩევანს განსაზღვრავს შემოვლითი და გამჭოლი განტოტებების არსებობა დეზობლიტერაციის აუცილებელ დონეზე.

ბარძაყის ღრმა არტერიის სტენოზის დროს 4სმ-მდე სიგრძით ფოლაქის სიმკვრივისაგან დამოუკიდებლად, რეკომენდებულია ღია ენდარტერექტომია აუტოვენური, აუტოარტერიული და/ან ალოჩანართით; სტენოზის 4-დან 10 სმ-მდე ზომის შემთხვევაში, რბილი ან საშუალო სიმკვრივის ათეროსკლეროზული ფოლაქით, ნაჩვენებია ღია ენდარტერექტომია აუტოვენური ნაკერით. 10 სმ-ზე მეტი სიგრძის სტენოზის დროს, ათეროსკლეროზული ფოლაქის სიმკვრივისგან დამოუკიდებლად, რეკომენდებულია ბარძაყის ღრმა არტერიის შუნტირება და/ან პროთეზირება. ბარძაყის ღრმა არტერიის პროთეზირებისას და გამჭოლი და/ან შემოვლითი არტერიების 2 მმ-ზე მეტი დიამეტრის შემთხვევაში, შენახული რეტროგრადული სისხლნაკადით, რეკომენდებულია მათი რეიმპლანტაცია.

10-ЛЕТНЯЯ ЛЕТАЛЬНОСТЬ У ПАЦИЕНТОВ ПОСЛЕ ПЕРЕЛОМОВ ПРОКСИМАЛЬНОГО ОТДЕЛА БЕДРЕННОЙ КОСТИ

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Переломы проксимального отдела бедренной кости (ППОБК) являются тяжелым, инвалидизирующим осложнением системного остеопороза, приводящим к повышению уровня смертности. Частота переломов данной локализации у пациентов пожилого и старческого возраста прогрессивно увеличивается в экспоненциальной зависимости, что связано с огромными финансовыми затратами на лечение и реабилитацию больных [12,14,15,17].

В различных исследованиях продемонстрировано возрастание показателей краткосрочной [1-4,6,7,9,11,13] и долгосрочной [8,18] летальности после ППОБК и проанализированы факторы [7,11,13,16,18], влияющие на показатели выживаемости больных. Летальность в стационаре обычно является невысокой (1,7-10%) и связана с лечебной тактикой, продолжительностью пребывания в стационаре и возникшими в послеоперационном периоде осложнениями [5,6,9,10]. Дополнительными неблагоприятными факторами, влияющими на уровень летальности, являются возраст больных и мужской пол. Наибольший риск смерти у больных после ППОБК регистрируется в первые полгода после перелома, а показатели летальности в течение года составляют от 18 до 35%, при этом большинство выживших пациентов ограничены в самообслуживании и требуют дополнительного ухода [1,6,7,10]. Своевременное оперативное лечение больных с ППОБК, в частности эндопротезирование тазобедренного сустава, способствует быстрой мобилизации пациентов после операции, более высокому качеству жизни и снижению показателей летальности.

В настоящее время продемонстрировано, что как частота ППОБК, так и показатели летальности имеют некоторые географические особенности, что обусловлено уровнем медицинской помощи, благосостоянием населения и другими факторами [14,15,17]. За последние годы в связи с более широким внедрением эндопротезирования тазобедренного сустава после ППОБК улучшился жизненный прогноз и качество жизни больных.

На сегодняшний день в Украине существуют единичные исследования, в которых проанализированы показатели ранней, в том числе и госпитальной летальности, у больных с ППОБК [1,2], однако более длительные наблюдения ранее не проводились, что и послужило основанием для проведения данного исследования.

Цель исследования – определить показатели кратко- и долгосрочной летальности у пациентов после переломов проксимального отдела бедренной кости.

Материал и методы. В ретроспективном исследовании проанализированы данные 228 пациентов, проживающих в г. Львов (146 женщин и 82 мужчины), госпитализированных в 2005-2007 гг. в травматологическое отделение коммунального некоммерческого предприятия «Клиническая больница скорой медицинской помощи» в связи с ППОБК. Данное исследование утверждено Этическим комитетом ГУ «Институт геронтологии им. Д. Ф. Чеботарева НАМН Украины» (29.01.2015, протокол №1).

Информация о демографических характеристиках больных, сопутствующей патологии, локализации перелома и особенностях его лечения проанализирована с использованием истории болезни. Данные о жизненном исходе собирались трижды – в 2015, 2016 и 2017 гг. посредством телефонного контакта с больными или их родственниками. При утере связи с пациентами в случае их смерти или отсутствия контакта с родственниками – по данным «Львовского городского отдела государственной регистрации актов гражданского состояния» с запросом о дате смерти пациента.

В исследование включены пациенты в возрасте 50 лет и старше (средний возраст 74,5 [64,7-80,8] лет), которые получили травму с ППОБК в быту (первичная госпитализация). Критериями исключения явились травмы вследствие дорожно-транспортных происшествий, патологические ППОБК на фоне онкологической патологии и более молодой возраст больных (менее 50 лет).

В последующем анализ проводили как в общей группе, так и в отдельных возрастных подгруппах (50-59, 60-69, 70-79 и 80-89 лет) в зависимости от пола больных, локализации перелома и тактики лечения.

Статистическую обработку полученных результатов проводили с использованием программы «Statistica 10.0». Проверка выборки на соответствие закону нормального распределения с помощью Shapiro-Wilk W-теста продемонстрировала характер распределения показателей, отличающийся от нормального, в связи с чем результаты представляли в виде медианы и нижнего и верхнего квартилей (Me [25Q-75Q]). Сравнение показателей двух независимых выборок проводили с использованием Mann-Whitney U-теста, оценку различия частот в двух независимых выборках – с помощью χ^2 критерия. Показатели летальности рассчитывали согласно стандартной формуле (отношение числа умерших с ППОБК за определённый период времени к общему числу лиц с ППОБК). Выживаемость пациентов оценивали с помощью анализа Каплана-Мейера, сравнение двух групп - с использованием Gehan's Wilcoxon теста. Различия показателей считали достоверными при $p<0,05$.

Результаты и обсуждение. Среди обследованных женщины составили 64%, мужчины - 36%, средний возраст мужчин был достоверно меньше (мужчины – 66,7 [57,4-78,4] лет, женщины – 77,7 [68,1-81,9] лет, $Z=4,7$; $p<0,0001$).

Средний период наблюдения за больными составил 121,3 [30,6-143,9] мес. (143,4 [133,4-150,0] мес. для выживших пациентов и 49,4 [10,2-120,3] мес. - для умерших).

Возрастное распределение обследованных в общей структуре пациентов с ППОБК продемонстрировало большую долю молодых лиц среди мужчин и лиц старших возрастных групп среди женщин, что подтверждает существующие данные о возрастных особенностях ППОБК [15,17]. Мужчины в возрасте 50-59 лет составили 32,9%, 60-69 лет – 31,7%, 70-79 лет - 17,1%, 80-89 лет – 18,3%. Соответствующие показатели у женщин составили 6,8%, 22,6%; 32,9% и 37,7%. У мужчин, как и у женщин, старших возрастных групп отмечена достоверно большая частота ($p<0,05$) ко-

морбидных состояний в сравнении с группой более молодых пациентов (50-69 лет).

Анализ локализации ППОБК у мужчин и женщин выявил некоторые особенности: у женщин наиболее часто переломы были внутрисуставными и локализировались в шеечном отделе (54,1%), в меньшей степени – внесуставными (чрезвертельные - 39,7% и подвертельные - 6,2%). У мужчин шеечные и чрезвертельные переломы регистрировались приблизительно одинаково - 40,2% и 47,6%, соответственно. Наиболее редкой локализацией у женщин были подвертельные переломы (12,2%), что согласуется с данными других исследователей [12].

Анализ тактики ведения пациентов показал, что оперативные методы лечения использованы у 53,6% мужчин (50% металлоостеосинтез и 3,6% эндопротезирование тазобедренного сустава) и у 58,5% женщин (53,7% металлоостеосинтез и 4,8% эндопротезирование тазобедренного сустава). Низкий уровень оперативной активности у пациентов, госпитализированных в период 2005-2007 гг., в отличие от показателей, зарегистрированных в данном лечебном учреждении в 2018-2019 гг. (62,4% у мужчин и 69,5% у женщин), очевидно, связан с более низким показателем внедрения операции эндопротезирования в предыдущие годы, отсутствием на тот момент государственных программ поддержки больных с ППОБК.

Средний показатель продолжительности госпитализации достоверно не отличался в зависимости от пола и составил 25,5 [12,5-43,0] дней (для мужчин – 24,5 [14-39] дней и 27,0 [12-48] дней для женщин).

Средний возраст при поступлении для выживших был достоверно меньше в сравнении с показателем умерших к концу исследования (67,9 [60-76,8] лет и 77,9 [67,4-82,7] лет, соответственно; Z=4,4; p<0,0001). Средний возраст на момент смерти для умерших (81,2 [72,2-85,1] лет) не отличался от такового выживших на момент окончания исследования (79,2 [72,8-89,4] лет).

Анализ показателей больных в зависимости от пола выявил, что среди умерших средний возраст на момент смерти составил 81,2 [72,2-85,1] лет и был достоверно выше у женщин (82,0 [72,9-86,8]) в сравнении с мужчинами (76,8 [66,3-84,8] лет; Z=2,0; p=0,04). Средний возраст выживших на момент завершения исследования составил 79,2 [72,8-89,4] лет и был достоверно выше у женщин (84,5 [76,9-91,3]) в сравнении с мужчинами (72,8 [67,7-80,1] лет; Z=3,8; p<0,0001).

Показатели госпитальной летальности отличаются в различных исследованиях, что, по всей вероятности, связано с тактикой ведения больных, сопутствующими осложнениями и другими факторами. Так, в исследовании R. Civinini [5] показатели больничной летальности составили 2,4%, согласно M. Gurger [6] – 11%. В исследовании украинских авторов, анализирующих показатели госпитальной смертности от ППОБК за 2011-2018 гг., показатель колебался в пределах от 5,5 до 22% в зависимости от года наблюдения [1]. В данном исследовании 2,6% пациентов среди умерших умерло в течение 30 дней, при этом показатель 30-дневной летальности составил 1,3%.

Наибольшие показатели летальности после ППОБК регистрируются в течение первого года после перелома, особенно в первые 6 мес., однако варьируют в широких пределах: в исследовании R. Civinini [5] показатели летальности спустя год после ППОБК составили 18,7%, в исследовании M. Gurger [6] – 22%, I.S. Cenzer [4] – 27%, а у O. Guzon-Illescas [7] – 33%. В проведенном ранее исследовании украинских авторов [2], которые проанализировали показатели летальности у пациентов после ППОБК в течение одного года, продемонстрировано, что наибольшие показатели летальности отмечались в течение первых 6 мес. после перелома; показатели 1-летней летальности составили 18,5%. По данным многих исследователей [2,4,7,11,13], возраст, мужской пол и наличие сопутствующей патологии являются отрицательными факторами, влияющими на показатели летальности после ППОБК. Авторы [6,11] отмечают значимую роль своевременности оперативного вмешательства у больных с ППОБК, а также наличие послеоперационных осложнений.

По данным настоящего исследования, в течение 6 мес. после перелома умерло 23,3% пациентов, спустя 12 мес. – 36,2%. Соответствующие показатели летальности составили 11,8% и 18,4%.

В проекте CHANCES (The Consortium on Health and Ageing: Network of Cohorts in Europe and United States) [8] с участием более чем 120 тысяч лиц из 8 когорт Европы и Америки изучены показатели летальности, в среднем, спустя 12,6 лет после ППОБК. Авторами показано, что ППОБК связан с повышением уровня общей летальности в течение указанного периода времени (отношение рисков (HR)=2,12; 95% доверительный интервал (ДИ) =1,76-2,57) и был несколько выше у мужчин (HR=2,39; 95% ДИ=1,72–3,31) в сравнении с женщинами (HR=1,92; 95% ДИ=1,54-2,39) без достоверных различий между группами. Показатели летальности были выше в течение первого года после перелома (HR=2,78; 95% ДИ=2,12-3,64) и оставались повышенными в дальнейшем без значительных колебаний в течение более длительного периода наблюдения (HR=1,89; 95% ДИ=1,50-2,37 спустя 1–4 года; HR=2,15; (95 % ДИ: 1,81-2,55) через 4-8 лет и HR=1,79; 95% ДИ=1,57-2,05 спустя 8 и более лет исследования).

Исследование с использованием данных шведского регистра (1013 пациентов с ППОБК 2026 лиц группы сравнения, находившихся под наблюдением в течение 22 лет) [18] продемонстрировало, что у мужчин и женщин различного возраста с ППОБК показатели летальности достоверно выше в сравнении с показателями лиц без переломов. Риск смерти (risk ratio, RR) в течение всего периода наблюдения составил у женщин 1,8 (95% ДИ=1,6-2,0), у мужчин – RR=2,7 (95% ДИ=2,1–3,3). Данный показатель уменьшался с 4,6 (95% ДИ: 3,5-6,1) через год после перелома до 2,2 (95% ДИ: 1,9-2,5) через 5 лет и до 1,9 (95% ДИ: 1,7-2,1) через 15 лет после ППОБК. Соответствующие показатели у мужчин составили 5,7 (95% ДИ: 3,7-8,9), 3,1 (95 % ДИ: 2,4-4,1) и 2,8 (95% ДИ: 2,2-3,5).

В проводимом исследовании в течение 5 лет от момента перелома умерло 73,0%, а через 10 лет – 95,7% пациентов. Соответствующие показатели летальности составили 36,8% и 48,2%, что подтверждает результаты других ранее проведенных исследований.

На момент окончания исследования умерло 64,5% пациентов (мужчины – 64,6%, женщины 64,4%). Показатели летальности среди мужчин в возрастной группе 50-59 лет составили 51,9%, 60-69 лет – 57,7%, 70-79 лет – 64,3% и 80-89 лет – 100,0%. Соответствующие показатели у женщин составили 40,0%, 48,5%, 65,3% и 77,8%. Показатели летальности не отличались у мужчин и женщин в возрастных группах 50-79 лет и лишь в группе 80-89 лет были достоверно выше у мужчин (χ^2=4,0; p=0,045).

В ранее проведенных исследованиях украинских авторов [3] продемонстрировано, что показатели летальности по-

сле ППОБК отличаются в зависимости от вида перелома и метода лечения. Среди умерших 26,4% были с чрезвертельными и подвертельными переломами, 73,6 - с переломами шейки бедренной кости [3]. В нашем исследовании достоверных различий показателей летальности в зависимости от локализации перелома не получено.

Анализ показателей выживаемости с помощью теста Каплана-Мейера больных с ППОБК представлен на рис. 1. Различий выживаемости в зависимости от пола, вида переломов и методов консервативного лечения достоверных различий между группами за исключением пациентов моложе и старше 70 лет (p = 0,004 согласно тесту Gehan's Wilcoxon, рис. 2) не выявлено.

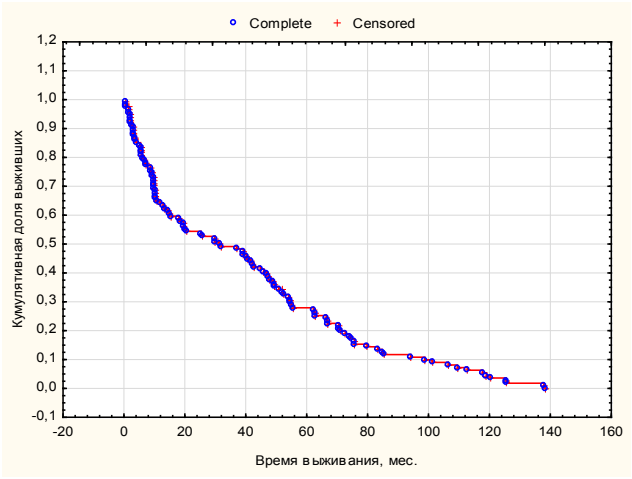

Рис. 1. Кривая выживаемости у мужчин и женщин с переломом проксимального отдела бедренной кости

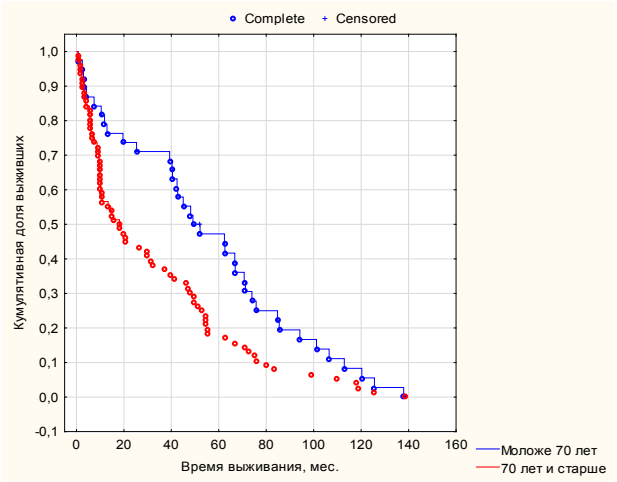

Рис. 2. Кривая выживаемости у пациентов с переломом проксимального отдела бедренной кости

Выводы. Долгосрочное изучение показателей летальности у пациентов с ППОБК выявило, что показатели госпитальной летальности составили 1,3%, 6-месячной, 1-, 5- и 10-летней летальности, 11,8%, 18,4%, 36,8% и 48,2%, соответственно. Показатели летальности у мужчин выше в сравнении с показателями женщин лишь в возрастной группе 80-89 лет, при этом достоверных отличий летальности в зависимости от вида переломов не выявлено. Показатели выживаемости не отличались в зависимости от пола, вида перелома и были достоверно выше (p=0,004) в группе больных старше 70 лет в сравнении с более молодыми пациентами.

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SUMMARY

10-YEAR MORTALITY IN PATIENTS AFTER HIP FRACTURES

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The aim is to study the indexes of short- and long-term mortality in patients after hip fractures (HF).

In a retrospective study, the data of 146 women and 82 men with HF aged 50 years and older (mean age (Me [25Q-75Q]): 74.5 [64.7-80.8] years old) hospitalized in 2005-2007 were analyzed. Life outcome data were collected three times (in 2015, 2016 and 2017) by the researcher by telephone contact with patients or their relatives. The analysis was carried out depending on age, gender, type of fracture, the presence of concomitant diseases. The average follow-up period was 121.3 [30.6-143.9] months (143.4 [133.4-150.0] months for surviving patients and 49.4 [10.2-120.3] months for deceased). Women accounted for 64 % of all subjects with HF and were significantly older than men. The average age at the time of death for the deceased (81.2 [72.2-85.1] years) was significantly higher in women (82.0 [72.9-86.8]) compared with men (76, 8 [66.3-84.8] years; Z=2.0; p=0.04), although it did not differ from the indexes of survivors at the end of the study (79.2 [72.8-89.4] years). Hospital mortality rates were 1.3%, 6-month, 1-, 5- and 10-year mortality, respectively – 11.8%, 18.4%, 36.8 \% and 48.2%. Mortality rate was higher in men only in the age group of 80-89 years, while there were no significant differences in mortality depending on the type of fracture. Survival rates did not differ depending on gender, type of fracture, and were significantly higher (p=0.004) in the patients older than 70 years compared with younger patients.

Keywords: hip fractures; short-term mortality; long-term mortality.

РЕЗЮМЕ

10-ЛЕТНЯЯ ЛЕТАЛЬНОСТЬ У ПАЦИЕНТОВ ПОСЛЕ ПЕРЕЛОМОВ ПРОКСИМАЛЬНОГО ОТДЕЛА БЕДРЕННОЙ КОСТИ

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Цель исследования – изучить показатели кратко- и долгосрочной летальности у пациентов после переломов проксимального отдела бедренной кости.

В ретроспективном исследовании проанализированы данные 146 женщин и 82 мужчин в возрасте 50 лет и старше, средний возраст - Me [25Q-75Q]:74,5[64,7-80,8] лет, госпитализированных в 2005-2007 гг. в связи с переломом проксимального отдела бедренной кости (ППОБК). Данные о жизненном исходе собирались трижды - 2015, 2016 и 2017 гг., посредством телефонного контакта с больными или их родственниками. Анализ проведен с учетом возраста, пола, вида перелома, наличия сопутствующей патологии. Средний период наблюдения за больными составил 121,3 [30,6-143,9] мес. (143,4 [133,4-150,0] мес. для выживших пациентов и 49,4 [10,2-120,3] мес. для умерших). Женщины составили 64% от всех пациентов с ППОБК и были достоверно старше мужчин. Средний возраст на момент смерти составил 81,2 [72,2-85,1] лет и был достоверно выше у женщин (82,0 [72,9-86,8]) в сравнении с мужчинами (76,8 [66,3-84,8] лет; Z=2,0; p=0,04), однако не отличался от показателя выживших на момент окончания исследования (79,2 [72,8-89,4] лет). Показатели госпитальной летальности составили 1,3%, 6-месячной, 1-, 5- и 10-летней летальности – 11,8%, 18,4%, 36,8% и 48,2%, соответственно. У мужчин в возрастной группе 80-89 лет показатели летальности были выше; достоверных отличий в летальности в зависимости от вида переломов не выявлено. Показатели выживаемости не зависели от пола и вида перелома и были достоверно выше (p=0,004) в группе больных старше 70 лет.

რეზიუმე

პაციენტების 10-წლიანი ლეტალობა ბარძაყის ძვლის პროქსიმალური ნაწილის მოტეხილობის შემდეგ

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კვლევის მიზანს წარმოადგენდა მოკლე- და გრძელვადიანი ლეტალობის მაჩვენებლების შეფასება პაციენტებში ბარძაყის ძვლის პროქსიმალური ნაწილის მოტეხილობის შემდეგ.

რეტროსპექტულ კვლევაში გაანალიზებულია 50 წლის და მეტი ასაკის, საშუალო ასაკი - Me [25Q-75Q]): 74,5 [64,7-80,8] წელი, 146 ქალის და 82 მამაკაცის მონაცემები, რომელნიც 2005-2007 წწ. ჰოსპიტა-

ლიზებული იყვნენ ბარძაყის ძვლის პროქსიმალური ნაწილის მოტეხილობის შემდეგ. მონაცემები სიცოცხლის გამოსავლის შესახებ შეკრებილ იყო სამჯერ – 2015, 2016 და 2017 წწ., სატელეფონო კონტაქტით პაციენტებთან ან მათ ნათესავებთან. ანალიზი ჩატარდა ასაკის, სქესის, მოტეხილობის სახეობის, თანმხლები პათოლოგიის არსებობის გათვალისწინებით. პაციენტებზე დაკვირვების საშუალო პერიოდმა შეადგინა 121,3 [30,6-143,9] თვე: 143,4 [133,4-150,0] თვე – გადარჩენილი პაციენტებისათვის, 49,4 [10,2-120,3] თვე – გარდაცვლილებისათვის. ქალებმა შეადგინეს ყველა პაციენტის 64% და სარწმუნოდ მეტი ასაკის იყვნენ მამაკაცებთან შედარებით. საშუალო ასაკმა გარდაცვალების მომენტისათვის შეადგინა 81,2 [72,2-85,1] წელი და სარწმუნოდ მეტი იყო ქალებში (82,0 [72,9-86,8]), მამაკაცებთან შედარებით (76,8 [66,3-84,8] წელი; Z=2,0; p=0,04), თუმცა, კვლევის დასრულების მომენტისათვის არ განსხვავდებოდა გადარჩენილთა მაჩვენებლებისაგან (79,2 [72,8-89,4] წელი.

საშუალო ასაკი გარდაცვალების მომენტისათვის იყო 81,2 [72,2-85,1] წელი და სარწმუნოდ უფრო მაღალი იყო ქალებში. ჰოსპიტალური ლეტალობის მაჩვენებელმა შეადგინა 1,3%, 6-თვიანი, 1-, 5- და 10-წლიანი ლეტალობისა – 11,8%, 18,4%, 36,8% და 48,2%, შესაბამისად. 80-89 წლის ასაკის მამაკაცებში ლეტალობის მაჩვენებელი მეტი იყო, ამასთან, ლეტალობაში სარწმუნო განსხვავება მოტეხილობის სახეობასთან დამოკიდებულებით არ გამოვლინდა. გადარჩენის მაჩვენებლები არ აღმოჩნდა დამოკიდებული სქესსა და მოტეხილობის ტიპზე და სარწმუნოდ უფრო მაღალი იყო (p=0,004) 70 წელზე მეტი ასაკის პირთა ჯგუფში.

STUTTERING: INITIATING FACTORS, EVOLUTION, HEALING PERSPECTIVES

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The stuttering represents the speech disorder caused by involuntary spasmodic contractions of articulation muscles, primarily of the vocal cord and the mouth upper lip. Due to unconscious twitches of the voice producing muscles, pronunciations of separate speech constituents under stuttering are delayed and disturbed, while of others are on the contrary hastened, but are disorders also. Throughout the total world population the stuttering rate in children approximates 10%, while 4% among preserves the complaint in adulthoods also [5]. According to the more contemporary statistics, about 1% of the general world population, mostly of children and adolescents, suffers from the stuttering [4], while 0.8% and 0.2% from are males and females, respectively [12]. 55 million subjects stutter worldwide in sum [2]. Professionally speaking, the stuttering is a symptom, but not a disease, although the term *stuttering* usually refers to both the symptom and the illness [2].

As stated by the American Speech-Language-Hearing Association [19], the major stuttering signs are as follow: (a) Adding up to the speech materials the sounds or words, labeled as interjections; (b) Repetition of word parts; (c) Repetition of one-syllable words; (d) Speech locks or stops; (e) Prolonged sounds; (f) Repetition of words; (g) Repetition of phrases; (h) Changes of words in sentences, labeled as revisions; (i) Uncompleted thoughts. The stuttering may be accompanied by other hints also, e.g. by the head nodding and the eye blinking. Being stutterer excited or feeling rushed, vocal muscle frustrations and/or tensions can exaggerate the speech hindrances further.

Rather similar set of guidelines were offered regarding the stuttering symptoms by the German Association of Otorhinolaryngology, Head and Neck Surgery [12]: (a) Repletion of sounds, syllables, one-syllable words; (b) Pauses between syllables within the words; (c) Lengthening of sounds; (d) Audible/inaudible speech blocks; (e) Repetition of words and/or phrases; (f) Uncompleted words; (g) Pauses in speech courses filled by extra-sounds or mutes; (h) Revision of words and/or utterances.

By the same German Association group [12] the stuttering escorted symptoms and psychological reactions to are outlined as follow: (a) Physical tensions; (b) Speech supplemented respiration changes; (c) Physical concomitants; (d) Speaking mode changes; (e) Speech avoidance efforts: preventive paraphrasing, rephrasing, attempts to substitute the feared words; (f) Insertion of sounds/syllables; (g) Insertion of words and phrases; (h) Conspicuous changes in communications; (i) Uncompleted sentences, repeated phrases, stop-and-go trials (recoil); (j) Avoidance of particular situations; (k) Fear, embarrassment, shame; (l) Vegetative reactions.

By the German Otorhinolaryngology, Head and Neck Surgery Association gathering [12] the stuttering covert symptoms are summarized into three points: (a) Avoidance of peculiar situations; (b) Emotional reactions and psychosocial stresses; (c) Cognitive reactions.

The etiology of the stuttering seems not completely clear till now [4]. The most of the stuttering signs are displayed up to the age of five years with about equal illness rates in both genders [20]. Recovery from the ailment is nevertheless about four times less in boys than in girls, that being attributed to the higher degree of language hemisphere lateralization in males vs. females [2]. The stuttering initiation is provided by the inner and/or the outer factors [1, 5]. The principal ailment determinants in children are [11]: (a) Genetic cues, when mother or father suffers or suffered earlier from the stuttering; (b) Complicated pregnancy and/or delivery that can affect the child's nervous system and can disturb its steadiness to the outer influencing agents; (c) Frequent and/or complicated somatic disorders in early childhood, which can exert exhausting effects upon the nervous system and can deteriorate the latter's stability to the irritants around;

(d) The deviant functioning of the nervous system that can prolong and disturb the phrase completion span and can retard consequently the speech formation process; (e) Regular supply of the child by parents and/or other social encirclement persons with complicated speech material that being hard to process, in general, for children of early ages, in particular; (f) The tense and conflicting family situations. The stuttering in adults can be associated with a substantial psychological morbidity including the low quality of life and the dropped social activity [13], although there were many stutterers, e.g. *Winston Churchill* and *Charles Darwin*, who became famous, despite the early-aged serious stuttering.

Developmental and acquired, by other terminology idiopathic and neurogenic stuttering types are differentiated [2]. The developmental stuttering involves mostly the children of 2-5 years of age without any apparent brain impairment or other known causes, while the acquired stuttering follows the definable brain damage, e.g. the stroke, intracerebral hemorrhage, head trauma [2, 16]. More than 5% of adult subjects exhibit after the stroke the neurogenic stuttering, while in about 3% the speech disorder persists during more than six months [16]. In rare cases the acquired psychogenic stuttering is initiated by the psycho-trauma or psychological illness [12]. The developmental stuttering, as compared with the acquired one, is particularly prominent at starting parts of words or phrases as well as with respect to extended words. Nonetheless, the distinctions between either stuttering types are not always simple, as far as they use to overlap each other [2].

The distorted basal ganglion activity in stutterers' brain and normalization of the shifts after the successive treatment were verified in previous studies [8, 18]. The hyperactivity of the lateral premotor cortex [3] and of the cerebellum [6] was also detected. The right hemisphere was additionally proved to be more excitable in stutterers than in healthy individuals [2]. It has been furthermore noted that the stuttering underlining mechanisms dominantly cover the temporal and frontal speech-language hemisphere centers as well as the motor and association premotor regions [2].

The listed as well as other similar factors can influence the child's nervous system and can intensify its reactivity to the outward signals. As a result, even scant irritant may become capable to create the neurotic disorders, generally, the stuttering, particularly. The stuttering prevention is more available before its setting up, while the problem negligence just during the pathology initiation phase can lead to more serious and steady disturbances [11].

Generating and supporting items of the stuttering are numerous. The irritants producing sensitive fears appear dominating among. The degree of the fear and the functional state of accepting nervous system are both involved in stuttering extent gradation. Under sensitive psychological background, the disorder can be initiated by the thunderstorm, heavy knocking on a door, loud shouting, a dog barking, to be lonely in a dark room [17]. Due to the indefinite reasons, the stuttering can arise instantaneously even.

Excitation and inhibition, two principal constituents of the nervous functioning are regularly substituting each other. The replacements accompany the speech processes also. When affected by the strong irritants, the neural reactions to are intensified that can violate the excitation/inhibition ratio, while just the stuttering may be the outcome of disproportions followed [14]. The respective case can be recalled from the own practice. The children in a group manner were returning from the school. The barking dog started suddenly to succeed them in an aggressive manner. All children were more or less frightened and began to run. Afterwards one from started to exhibit the stuttering. When inquiring the parents, it was ascertained that the specific emotional predisposing factor had already affected this child previously. Due to this reason, just he appeared to possess the heightened risk for the stuttering origin.

The imbalance in coupled excitation/inhibition ratios can degrade the nervous functioning. It can particularly happen when the subject tries to suppress something in own feelings and to hide the restraints from others. A relevant example from the private practice is presented beneath. The little boy had no positive respects to the stepfather and avoided any contacts with him. However he tried to hide his negative attitudes from surroundings as far as had the sense that the denying outlooks hurt his mother emotionally. Due to perpetual accumulation of negative emotions and hindrances in their expression, the stuttering was developed in this child later, at the age of ten years.

The psycho-disturbances can lead to the neurotic state that can per se initiate the stuttering. The respective example may be reminisced from the private practice also. The mother wanted to lay the child for a sleep in a bed, while the grandmother insisted to take him outside for the pre-sleep walk. The child was confused in decision: to the mother or the grandmother has he to obey. The stuttering followed to his stressful state.

Another occasion can be recalled from the own experience. The child was left-handed, while the parents insisted to correct his faulty likely habit. Correspondingly, they forced him in impelling situations instead of the favored left hand to utilize the less dominating in this concrete case right one. In right-handed majorities the left hemisphere, while in left-handed minorities the right hemisphere is prevailed, with respect to the speech function particularly. When the left-handed person is appealed to use the non-preferred right hand instead of the favored left one, he is forced to affect the dominance of the right hemisphere speech function and to realize the relevant duties through the leader participation of the left hemisphere, that being less adapted for speech just in left-handed individuals. Under such peculiar stressful situations dyslexia and dysgraphia may be created in some left-handed, i.e. right-hemisphere dominant individuals, while the stuttering can occur in others. Just stuttering was followed to the outward situation in the commented case.

The peculiar stuttering type has an imitative character. Some emotional children mostly unconsciously mimic the stutterers around. If the child's parents are not disorder-carrying individuals, hereditary and imitative stuttering variances are not easy to distinguish.

Genuine and provocative stuttering types are also hard to discern, while in some individuals delimitation is impossible even. These two stuttering variances are usually interrelated, the dominating effect of any one against another being better manifested at initial pathology stage mostly.

The stuttering covers physical and psychological symptoms [17]. The physical set includes the contractions of speech muscles, i.e. the principal stuttering outlooks. Under contractions, the muscles exhibit momentary or prolonged convulsive twitches, just to which the weakened and/or delayed and/or suspended speech is associated. The convulsions may persist tens of seconds, while in severe cases their length can approximate to the minute span even.

The stuttering associated muscle contractions are of a tonic or a clonic character. The tonic twitches are more intricate stuttering attributes than the clonic ones. The convulsions of isolated

types happen however rare: mostly they have the tonic-clonic, i.e. the mixed, but predominantly of the tonic, or the clonic-tonic, i.e. also the mixed, but predominantly of the clonic character. Convulsions of both groups involve speech and respiratory muscles in the main globally. Therefore, they participate in stuttering composition mostly in combined manner, while in rare cases separately also.

Under heightened emotional background, the speech concomitant involuntary muscle contractions may concern the non-speech structures also: head, neck, shoulders, hands, legs, body. Stuttering associated non-speech muscle contractions are caused by spreading of excitation from speech muscle hemisphere center to the neighbor areas, representing the muscles just of mixed and/or non-speech natures [17]. The speech concomitant mixed and/or non-speech muscle contractions further complicate the stutterer's state.

Psychological consequences of the stuttering are of an individual character. They use to include mental misbalances, negative emotions, inferiority complex, loneliness feeling, pessimistic mood, and difficulties in contacts with others, including the family members as well as the crèche and/or the kinder-garden and/or the school mates.

The stuttering symptoms are partly conditioned by alterations of the vegetative nervous system. Under various neural disorders, including the stuttering, the governed influence of the hemisphere cortex upon the vegetative nervous system can weaken, that may be followed by the respective complaints. The vegetative dysfunctions under stuttering are primarily manifested in arterial pressure rise and heart rate acceleration as well as in an excessive sweating. Along with intensification of muscle convulsions, the vegetative disorders are enhanced and widened in parallel. By the mechanism of the reversed influence the vegetative dysfunction can exaggerate in back the muscle twitches. The listed stuttering manifestations occur at starting pathology phases already. Just they have to judge therefore as the leader stuttering signs [17].

Along with dysphonia, many stutterers hold logophobia, the fear to the own speech processing. It can be associated with all verbal constituents or with particular vowels/consonants in. The fear to the speech actions usually accompanies the vegetative disorders. It further complicates the sufferer's state. In some stutterers the obsessive ideas are evanescing, while in others are permanent that tortures the holder's mood. The stuttering dramatically modifies the child's temper. Many stutterers avoid speech contacts with others, while some reject the relationships of any kinds. The stutterer child is mostly an introvert, plays alone, and violates the contacts with healthy mates even. The accessary emotions may result in more tense psychological distresses than the stuttering itself. All related negative psycho-feelings directly or throughout are nevertheless just the stuttering outcome.

According to the disorder manifestations, the stutterers are divided into three groups [15]. In associates of the first sample the pathology is exhibited in muscle convulsions solely, while no evidence of logo-neurosis exists at all. The stutterers of the second group, besides speech muscle contractions, endure logophobia but of the moderate degree only. The stutterers of the third species along with muscle convulsions suffer from sharply expressed obsessive fears. Herewith, they do not believe in healing chances and are correspondingly pessimistic with respect to the own ailment future.

Both physical and psychological stuttering symptoms are usually of long-lasting negative dynamics. The cure manager has not to wait however for the pathology endpoint and has to start an active treatment immediately, up to the accomplishment of inherent symptoms.

Considering the genuine factors, the stuttering is divided into the neurotic and the neurotic-like types [10]. The stuttering of the neurotic type arises on the background of psychological disorders. Before the event manifestations, the involved subjects are characterized by high sensitivity, while are suffered from disturbed sleep and appetite lack. In labile neural state persons the stuttering provocative factor can be the faint psychotrauma even. The initial ailment signs can be complicated by additional symptoms, e.g. by phobias and over-pessimistic estimations of own problems. In most sufferers the stuttering associates are intensified sometimes, while are weakened afterwards. Such a wavy course is more characteristic for the neurotic stuttering type.

The neurotic stuttering corresponds to the labeling by manifestations only: via the specific diagnostic approaches the brain lesion is confirmed just in these stuttering type patients [9]. The pathology essence is also validated by the anamnesis, particularly by indication on delayed speech commencement as well as on mother's toxic pregnancy and/or complicated delivery. Such children usually begin to speak belatedly, after the age of three years only.

The coupling of the neurotic stuttering with any definite factor is mostly difficult and conditional. The pathology has usually the slow course, while the remissions are not characteristic for [15]. Due to the organic brain lesions, the neurotic stuttering, as compared with the non-neurotic one, requires more argent and more qualified service.

The stuttering demands the team examination and estimation. Speech therapist, audiologist, psychologist, neurologist, psychiatrist are warranted to take part in an inspection process. The survey has to start with an anamnesis collection. Information has to get on mother's pregnancy and the delivery course. It has to ascertain, when the stuttering was initiated and what could be its reason. Taking into account the generating factor, it has to decide, whether the stuttering is of the neurotic or the neurotic-like type. The data have to acquire regarding the child's life conditions, routine daily behavior, relations between family members. The collected details promote the adequate planning of the pathology cure. When gathering the anamnesis, it has to learn what kind of treatment has been fulfilled previously and how successful its results were. The stutterer's attitude to the own problems has to establish in parallel. At the next stage, the sufferer's speech status is defined and the type of the muscle spasms is estimated. It is ascertained, whether the unintentional muscle contractions are associated with a speech and whether the stutterer is hurt from phobias. Particular attention has to draw to speech fluency and rhythms. The articulation state has to determine under loud and expressive readings also. When analyzing the acquired data, the stutterer's personal characteristics have to take into account also. Considering the results of the global inspection, the adequate habilitation/rehabilitation strategy is delineated.

The stuttering therapy aims to reduce the accompanying disturbances, to cancel the psycho-emotional stresses, to improve the stutterer's life quality, to expend the social involvements.

Among stuttering cure methods the composite approach is validated nowadays. Under the treatment processes, the pathology is considered as a compound speech disorder that being related with stutterer's nervous functioning, personal features, and life conditions. Habilitation/rehabilitation procedures include

the pedagogical and medical means aiming the recovery of the whole organism, while predominantly of the nervous system. Psychotherapy, speech therapy, optimization of social environments, improvement of life conditions are the principal items of the utilized procedures. Regular psychological impacts are also required. The stuttering in adults can be associated with substantial psychological morbidity including the low quality of life and the dropped social activity [13]. Both for stutterer children and adults the speech therapy appears the recovery mainstay [7, 13].

Affecting on the stutterer's nervous system is essential within the general therapy outlooks also. Due to the blockage of associated neurosis and of vegetative dysfunctions, the stutterer's speech status is improved, the inner calmness is strengthened, and the mood is stabilized. The stutterer begins to believe in own betterment. The treatment covers either physiotherapeutic approach and medicament therapy and is managed preferentially by the neurologist in a close cooperation with the specialists of relative topics as well as with the stutterer child's parents.

The peaceful family conditions are saliently important: under the tense household situations the cure results are delayed, appear less positive or totally unproductive even. In normalization of the nervous activity the important targets seem to be the selection of optimal working and resting balance, quiet night sleeping, systematic walking on a fresh air, and regular involvement in sports, preferably in their less emotional disciplines, e.g. the cycle racing, running, swimming. Organization of the child's relevant lifestyle without an active participation of parents and of other family members is difficult or impossible.

The stuttering associated somatic disorders negatively affect the stutterer's nervous system that can be followed by the speech function worsening. Stuttering blockage chances are reduced respectively. Prompt and regular treatment of the stuttering concomitant disorders is essential thus, that has been naturally executed by the specialists of the relevant topics.

Psychotherapy aims to alter the stutterer's attitude to the personal speech defects, to overcome the inferiority complex, to feel the own person as a faultless individual. Elimination of psycho-disorders and of phobias has to succeed primarily. Under reducing or disappearance of the phobias the speech muscle convulsions are gradually weaken, become rare, or disappear even. Just the blockage of psychological shifts and the abolishment of phobias are the principal targets of stuttering treatment efforts utilized.

Psychotherapy of stutterers covers rational/reasonable and inspirational/suggestive fields. In explanation/conviction form the rational approach is focused upon the influence on stutterer's mentality. The sufferer is provided by the information on convinced or supposed reasons of the pathology and on planned treatment procedures. The aimed approaches are explained in details. The significance of the stutterer's attitude to the personal problems as well as of the efforts for beneficial treatment output is emphasized. Psychotherapy intends to influence the stutterer's awareness. Hypnosis is also considered as the substantial psychology supporting item. The speech therapy is the principal ingredient of the cure service that is realized by logo-therapist in a close cooperation with sufferer child's parents, remainder family members, and other persons around. Optimization of coordinated functioning of the stutterers' vocal, articulatory, and respiratory systems is the primary aim of the speech habilitation/rehabilitation means applied. In speech medication trials the special attention has to draw to unforced inclusion of voice, peaceful uttering of words, and proper regulation of breathing. Under speech education trials the stutterer child is guided to accept logo-exercises. A healthy speech that is achieved via qualified approach should be consolidated by regular repetitions. As a result, the stutterer begins the systematic handling just of the mastered speech.

The treatment of the stutterer child via phonic exercises has to start through pronouncing of particular speech materials by the logo-therapist and the stutterer simultaneously. The imitative approach is utilized then. The regular rhythmic speech is inculcated at last. Weakening, rarefaction, and overcoming of speech spasms in the process of logo-exercises offer the positive psychological influence on the stutterer and prepare the basement for further cure achievements. The stutterer should regularly be involved in loud and expressive readings of particular texts. The respective procedures can naturally be applied in those cases only in which the stutterer child is already familiar with reading experiences. As far as the speech rate and the rhythm are disturbed primarily under the stuttering, in utilized habilitation/rehabilitation procedures particular attention has to draw to normalization just of logo-rates and logo-rhythms. Successes achieved in speech exercises have to extend systematically over everyday life situations.

The selection of an adequate tactic for the stuttering therapy is especially valid at starting treatment stages. The proper choose of restore means is particularly important under rapidly and sharply developed stuttering that being mostly the consequence of psychotraumas. In such cases, the speech organ of the stutterer child has to offer regular relaxation pauses. Consequently, the parents should be instructed at distinct initial management time spans to ensure the speech isolation periods of the stutterer child.

Various medicaments are employed for the stuttering treatment. The ailment was confirmed to be relieved under administration of neuroletpics, e.g. *haloperidol, risperidone, olanzapine* [2]. *Lidcombe* therapy was proved to have conspicuous effects in preschool children, while no indication exists until now for any favorable medication for pupils aged 6-12 years [12]. Rather high stuttering defeat rates are indicated in modern reviewing essays [20].

The providing of a stutterer child with peaceful social environments is particularly essential for the illness conquest. Correspondingly, the speech therapist has to involve in utilized affairs the sufferer child's parents as well as other family members. Regular efforts for establishment of stutterer's quite life conditions have to continue over all long-lasting treatment period.

A set of habilitation/rehabilitation procedures should be realized in each stutterer. The speech that is acquired under non-adequate treatment procedures is often forgotten soon. In such cases, the speech muscle twitches happen to reappear, while under vigorous manifestations even.

The stuttering blockage procedures have to fulfill continuously. The intervals between should be 20-30 days. During the pauses, the exercises executed by speech therapist have to continue under home conditions. In such combined situations the child's speech happens to restore significantly. Discontinuation of treatment affairs can result in the stuttering renewal. The evidence does not support the efficacy of pharmacotherapy, rhythmic speaking, breathing regulation, hypnosis as isolated stuttering treatment forms: just the combined application of various, while reasonable means provide the definite chance in attainment of optimal cure results [12].

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SUMMARY

STUTTERING: INITIATING FACTORS, EVOLUTION, HEALING PERSPECTIVES

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Despite high amount of incidents, no scientific paper existed up to now in Georgia dealing with the stuttering. In present essay the views over are collated. It is confirmed that the phenomenon reflects the speech rate and/or the rhythm distortions created by convulsive type involuntary contractions of voice-producing muscles. The disorder is either congenital or acquired. Complicated pregnancy and/or delivery, heavy and/or recurred somatic diseases, speech-formation delay, conflicting social situations appear the main provoking/supporting factors of. The stuttering covers physical and psychological symptoms. The physicals are manifested in speech muscle twitches, while the psychological in phobias. Neurotic and neurotic-like stuttering types are differentiated. The neurotics arise on the background of psychological disorders, the linkage of the neurotic-likes with any concrete factor being mostly difficult or impossible. It is emphasized that the stuttering treatment demands the complex application of pedagogical and medical means and aims the cure of the whole organism, while predominantly of the nervous system, and improvement of mode-of-life conditions of the sufferer. The necessity of the cure of associated diseases is emphasized. It is stated that the stuttering psychotherapy implies the blockage of mental disturbances, while the speech recovery trials intends the establishment of adequate voice, articulation, and respiratory functions. In utilized habilitation/rehabilitation means the particular attention has to draw to initiation of well-balanced logo-rhythms. The regulation of hemisphere speech-center function is a primary target of the vocal exercises applied. Achievements attained in study sessions are regularly spread over the vital situations. The favorable social environment is also regarded as an important item for the pathology defeat. The significance of the systematic cure interventions is emphasized the frequent and/or long-term pauses between being judged as the cause of habit remissions happened. Just compound and customary treatment and active involvement of parents and other family members in applied efforts ensure the better chances for the positive care output.

K**eywords:** Stuttering, initiating factors, pathogenesis, manifestations, evolution, treatment strategy, habilitation/rehabilitation means, healing perspectives.

РЕЗЮМЕ

ЗАИКАНИЕ: ПРОВОЦИРУЮЩИЕ ФАКТОРЫ, ТЕЧЕНИЕ, ПЕРСПЕКТИВЫ ИЗЛЕЧЕНИЯ

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Несмотря на широкую распространенность, по сей день в Грузии не было научной публикации по заиканию. В представленной работе охарактеризована данная патология. Поясняется, что заикание отражает нарушения темпа и ритма речи, продуцируемые конвульсивного типа непроизвольными сокращениями артикуляционных мыщц. Подчеркивается, что феномен бывает наследственным или приобретенным. Перечислены факторы, провоцирующие недуг: осложнения беременности и/или родов, тяжело протекающие и/или частые соматические заболевания, отставание в формировании речи, конфликтные ситуации в семье. Симптомы заикания делятся на физические и психологические. Физические проявляются в спазмах мышц, вовлеченных в речеобразовании, психологические - в фобиях. Дифференцированы невротические и неврозоподобные типы заикания. Невротические возникают на фоне психических

расстройств, связать же неврозоподобные с каким-либо конкретным фактором сложно или невозможно. Подчеркивается, что лечение заикания требует применения педагогических и медицинских средств и предусматривает психотерапию, улучшение бытовых условий пациента и санацию всего его организма, в первую очередь, нервной системы. Декларирована необходимость лечения сопутствующих заболеваний. Заявляется, что психотерапия направлена на блокирование когнитивных сдвигов, целью же логопедии является обеспечение синхронного и корректного функционирования голосового, артикуляционного, дыхательного аппаратов заики. Особое внимание уделяется регулированию логоритмики. Задачей речевых упражнений объявляется нормализация активности слухо-речевого мозгового центра. Достижения, зафиксированные в логосеансах, распространяются на жизненные ситуации. Указывается, что существенным моментом преодоления недуга является создание благоприятной социальной среды вокруг заики. Подчеркивается важность систематики лечебных процедур: частые и/или длительные межпроцедурные паузы могут стать причиной ремиссии патологии. Именно планомерное лечение и вовлечение родителей и других членов семьи в процессы габилитации/регабилитации являются основными факторами желаемого исхода.

რეზიუმე

ენაბლუობა: გამომწვევი ფაქტორები, მიმდინარეობა, განკურნების პერსპექტივები

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ენაბლუობის ფართო გავრცელებულობის მიუხედავად, სადღეისოდ საქართველოში არ ყოფილა გამოქვეყნებული სამეცნიერო ნაშრომი ამ თემატიკაზე. წინამდებარე პუბლიკაციაში პათოლოგიის შესახებ არსებული შეხედულებები არის შეჯერებული და მრავალწლიანი საკუთარი გამოცდილება განზოგადოებული. განმარტებულია, რომ ენაბლუობა სამეტყველო აპარატის კუნთთა კონვულსიური ტიპის უნებლიე შეკუმშვებით ინიცირებულ მეტყველების ტემპისა და რიტმის დარღვევებს წარმოადგენს. ხაზგასმულია, რომ ფენომენი თანდაყოლილი შეიძლება იყოს და შეძენილი. ჩამოთვლილია ენაბლუობის მაპროვოცირებელი ფაქტორები: გართულებული ორსულობა და/ან მშობიარობა, მძიმედ მიმდინარე და/ან ხშირი სომატური დაავადებები, მეტყველების განვითარებაში ჩამორჩენა, დაძაბული სოციალური გარემო. მინიშნებულია, რომ ენაბლუობის ფიზიკური სიმპტომოკომპლექსი მეტყველების პროცესში ჩართულ კუნთთა სპაზმებს, ხოლო ფსიქოლოგიური – ფობიებს მოიცავს. დიფერენცირებულია ენაბლუობის ნევროზული და ნევროზისმაგვარი ფორმები. ნევროზული ფსიქოდარღვევების ფონზე ვითარდება, ნევროზის მაგვარის დაკავშირება რომელიმე კონკრეტულ ფაქტორთან ხშირად რთული ან შეუძლებელია. ხაზგასმულია, რომ ენაბლუობის მკურნალობა სასწავლო-პედაგოგიურ და სამედიცინო პროცედურათა ჩართულობას, მთელი ორგანიზმის, უპირველეს ყოვლისა, ნერვული სისტემის სანაციას, ფსიქოთერაპიას, ენაბლუს ყოფითი პირობების ნორმალიზაციას ისახავს მიზნად. დეკლარირებულია ენაბლუობასთან ასოცირებულ სომატურ დაავადებათა მკურნალობის აუცილებლობა. გაცხადებულია, რომ ლოგოპედია ენაბლუს სახმო, საარტიკულაციო, სასუნთქ სისტემათა რაციონალური მოქმედებების ფორმირებისკენ, ხოლო ფსიქოთერაპია - მენტალურ დარღვევათა ბლოკირებისკენ არის მიმართული. ჰაბილიტაციის/რეჰაბილიტაციის პროცესში ლოგორიტმიკის კორექციას ექცევა განსაკუთრებული ყურადღება. ვოკალური ვარჯიშები მეტყველების სისტემის ჰემისფერული წარმომადგენლობის ფუნქციის ნორმალიზაციას ემსახურება. ლოგოსეანსების მიღწევები ცხოვრებისეულ სიტუაციებში გადაიტანება თანდათან. მითითებულია, რომ ენაბლუობის ბლოკირების პროცესში არსებით მომენტს კეთილგანწყობილი სოციალური გარემოთი ენაბლუს უზრუნველყოფა წარმოადგენს. ხაზგასმულია მეტყველების საჰაბილიტაციო/სარეჰაბილიტაციო ღონისძიებათა უწყვეტობის მნიშვნელობა: პროცედურებს შორის ხშირ და/ან ხანგრძლივ პაუზებს პათოლოგიის რემისიები შეიძლება სდევდეს თან. დადებით შედეგთა მოსაპოვებლად მნიშვნელოვან ფაქტორს პროცედურების კომპლექსთა სისტემატური მოხმობა და მოხმობილებში მშობელთა და ოჯახის სხვა წევრთა აქტიური ჩართულობა წარმოადგენს.

ANALYSIS OF IMMUNE CHANGES AND THEIR ROLE IN THE DEVELOPMENT OF ORAL AND OROPHARYNGEAL CANCER

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Cancer of the oral cavity and oropharynx is an immunosuppressive disorder. In its etiopathogenesis there is a fundamental insufficiency of immune surveillance, that is, the ability to recognize tumor cells as abnormal and destroy them before they build an obvious malignant tumor [15]. Due to the suppression of the immune system, tumor cells avoid recognition and lysis of tumor antigen (TA) by cytotoxic T-lymphocytes (CTL) of adaptive immunity [17]. In such patients there is a decrease in the absolute number of lymphocytes, disturbance of natural killer cellular activity (NK) [13], spontaneous apoptosis of cytotoxic T-lymphocytes [13], unsatisfactory antigen-presenting function [14].

Tumor cells can initiate and develop various mechanisms to avoid the body's immune response. Typically, this is one of the following mechanisms [10, 23]:

1. A resistance formation to apoptosis with variable expression of Fas-ligand (FasL), which leads to the death of tumor-infiltrating lymphocytes (TILs).
2. Producing of immunosuppressive molecules such as the transforming growth factor TGF-β, prostaglandin (PG) E2 and adenosine.
3. Loss of expression of co-stimulating molecules or cytokines, such as IL-6 and IL-10 interleukin.
4. A defect in the expression of antigens on the surface of the tumor cell, because antigens remain unidentified.
5. A reduction or loss of the molecule expression of the main complex of histocompatibility (MHC) class I and selective loss of human leukocyte antigen (HLA) and histocompatibility molecules (MHC) required for the interaction between TA and TA-specific CTLs, especially if there is low IFN-α regulation [14].

The violation of processes or insufficient recognition and TA processing is performed in tumor cells and in dendritic cells. An important role in these processes is played by the protein of the STAT family (signal transducer and activator of transcription). Reducing the amount of activated STAT1 contributes to lowering the expression of human leukocyte antigen (HLA) and MHF [16]. HLA are the proteins of the molecules of histocompatibility (MHC), which appear with antigen and are recognized by T-lymphocytes. Antigen-presenting molecules (antigenic peptide transporters – TAP1, TAP2; proteins – calnexin, calreticulin, ERp57 and tapasin) play an important role in the ability of MHC Class I molecules to migrate to the cell surface and to represent peptides of CD8+ T-lymphocytes, that is, to carry out the presentation of antigen [6, 13]. Reducing the regulation or loss of the expression of HLA molecules I class or other components, representing an antigen is one of the mechanisms for avoiding the immune response of the tumor. Lowering the MHC level class I is due to ganglioside, which is produced by cancer cells. So that, the tumor uses various mechanisms to induce changes in the system and to avoid an immune response to its development [25].

The role of dendritic cells in the development of oral and oropharyngeal cancer.

Dendritic cells (DC) that are the guards of the immune system are specialized, the strongest antigen-presenting cells (ARCs) and tolerant mediators [18, 20]. It is known that dendritic cells are divided into myeloid (MDC) and plasmacytoid (PDC). The first ones are the Langerhans cells contained in the epidermis and the mucous membrane of the upper digestive organs and breathing and thermal/interstitial MDCs that are in the dermis. Plasmacytoid DC of lymphoid origin are found in the blood and lymphoid organs.

DC mature in the presence of microbial products and inflammatory mediators such as TNF-a, IL-1 and IL-12. Mature DCs increase the regulation of other co-stimulating molecules - CD86, CD80 and CD40, as well as cytokines such as TNF a, IL-1 and IL-12. Subsequently, they pass through the bloodstream towards the lymph nodes and represent antigens, which they captured in peripheral tissues, T-lymphocytes [20], stimulating their differentiation into cytotoxic CD8 + cells capable of killing tumor cells [13].

MDC force tumor cells in the oral cavity and oropharynx to secrete immunosuppressive cytokines such as IL-1 and IL-10 [10]. IL-10 and TGF-β transform immature DCs in mature ones and at the same time tolerant to the antigen, which induces antigen-specific T cells, by activating Tregs and differentiating naïve CD4+ T-cells in Tregs.

Langerhans cells bind antigens to the flat epithelium and migrate back to the regional lymph nodes and/or other secondary lymphoid organs where they stimulate naïve T-lymphocytes. They can also represent antigens of T-lymphocytes memory to stimulate a secondary immune response [13]. A significant role of dendritic cells, in particular, Langerhans cells in the immune response to the development of oral cancer and oropharyngeal cancer, is confirmed in studies.

Increased concentration them in blood correlates with a positive prognosis and a greater survival of patients with oral and oropharyngeal cancer, and a decrease in metastasis in the cervix of the lymph nodes [13].

Cancer cells of the oral cavity and oropharynx with the help of tumor-associated fibroblasts stimulate the development of hepatocyte growth factor (HGF) [15], which inhibits the maturation of dendritic cells [19] and, relatively, the reduction of immune responses. Violations of monocyte chemotaxis and the ability of DC to form a cell cluster also reduce the protective response of the organism [25].

The role of cytokines in the development of oral and oropharyngeal cancer.

Different cells of the immune system provide a complex defense system with effective cytokine bindings. Cytokines are interleukins, interferons, tumor necrosis factor, growth factors and chemokines regulate cellular growth, proliferation, migration, signaling, both in tumor cells and in immune cells. In addition, the inflammatory component of the micro-environment of the tumor in the oral cavity and oropharynx is important in the immune surveillance and a response as its main component is cytokines. The micro cavity of the tumor of the oral cavity and the oropharynx is characterized by an unbalanced cytokine profile, which is dominated by immunosuppressive cytokines. It is also important to consider that the tumor cells of the oral cavity and the oropharynx independently produce anti-inflam-

matory cytokines that correlate with the survival of such patients [2]. Cytokines particularly cause an increase in the transmission signal and the activator of transcription of phosphorylation and phosphorylated extracellular signal-regulated kinase in patients with oral and oropharyngeal cancer [13].

Due to the secretion of chemokines and colony-stimulating factor (CSF)-1, cancer cells accumulate tumor-associated macrophages (TAMs) in their microenvironment, which create a favorable environment for developing of the tumor and avoiding the immune response by secreting TGF-β1, interleukin-6 (IL-6) and prostaglandin-E2 among other immunosuppressive cytokines [25].

According to several studies, patients with oral and oropharyngeal cancer have an imbalance of Th1/Th2 types of cytokines and increased Tregs [8,18]. Among the Th1 types of cytokines, IFN-γ, IL-12, TNF-α, whose levels are reduced, and Th2 cytokines, IL-4, IL-10, are elevated, while in healthy people the opposite result is observed [6].

It is investigated that the level of synthesis of soluble IL-2 receptor activated by T-cell antagonism of IL-2 is higher in patients with oral and oropharyngeal cancer, which is associated with an increased probability of the metastasis development and low overall survival [25]. IL-4 levels have also been significantly elevated, however, they have not confirmed the association of their overexpression with the tumor stage [22].

Elevated levels of IL-10 in serum of patients with oral and oropharynx cancer are correlated with an unsatisfactory prognosis of the treatment and are independent indicators of low survival [8]. In addition, such inflammatory cytokines as IL-6, HGF and VEGF are also determined in high serum concentrations and correlate with the recurrence [1].

In diagnosed patients with oral and oropharyngeal cancer, an increase in the level of IL-18 was observed. This cytokine is mainly synthesized by macrophages. Its role for cancer-ill patients is to stimulate the production of IFN-γ by NK cells and T-cells, which provides the immune response of the body [25].

In the environment of cytokines there is a production violation of pro-proliferative, immunosuppressive cytokines with the help of macrophage-associated tumors (TAM), unbalanced Hepatocyte Growth Factor (HGF) by fibroblast-associated tumor (TAF) as well as unbalanced STAT1/STAT3 signaling within tumor cells. The deficiency of tumor STAT1 signaling results in low production of chemokines CCL5 and CXCL10, and those chemokines that involve effector T-cells in the microenvironment of the tumor [16]. Excessive signaling pSTAT3 increases the production of cytokines that negatively regulate proinflammatory signals of danger, mature dendritic cells and cytolysis with natural killers and CTLs, and more specifically IL-6, IL-10, TGF-β1 and VEGF [15,16]. Compared to the control group, in patients with oral and oropharyngeal cancer, a 5-fold increase in TNF-α cytokine in blood [3].

The Tumor microenvironment.

The favorable conditions for the cancer growth of the mouth and the oropharynx are the conditions of the tumor microenvironment, which contains immune and stromal cells.

Cytokines, chemokines, T-cells, macrophages, dendritic cells, and natural killer cells (NK) are intracellular regulators of the immune cells activation. All of them are important participants in the formation of the tumor microenvironment. Any of their functional changes or their inhibition, affects the immune system response. For example, when there is an imbalance in the signal exchange system through cytokines, then tumor cells develop mechanisms to avoid the inhibitors growth of cytokines presented in the microenvironment of the tumor [13].

An important key to the progression of cancer is the loss of cellular connections and cellular polarity, called epithelial-mesenchymal transition (EMT). There are the factors that influence the process of EMT. It is promoted by the hypoxic environment and changes in the expression of the miRNA, which leads to a decrease in the regulation of E-cadherins (membrane protein CDH) [11]. Endothelial cells that secrete Bcl-2, an apoptotic regulatory protein, increase EMT-related changes by secreting IL-6, an important mediator of the acute phase of response.

EMT process in a tumor acquires migratory and invasive properties. In general, these changes can increase the metastatic potential of oral cancer and oropharyngeal cancer [25].

A malignant tumor of the oral cavity or oropharynx directly suppresses the immune response by developing mediators such as vascular endothelial growth factor (VEGF), prostaglandin E2 (PGE2), TGFβ, IL-6, and IL-10 [13]. Tumor cells also release pro-inflammatory mediators, including such a receptor as the IL-15 alpha subunit (IL15RA). In combination with IL-15, it provides enhanced synthesis of pro-inflammatory cytokines – IL-6, TNF-α and IL-17, which affects the immune response and as a result a low survival prognosis [10].

The role of T-cells in the antitumor immune response.

Stem cells in the bone marrow are the source of immature T-lymphocytes, which mature then in the thymus and migrate to the secondary lymphoid organs (lymph nodes, a spleen) as a naïve T-cell. ARCs represent antigens, by activating T-lymphocytes, after signaling of co-stimulatory molecules and binding of MHF to ARCs and T-cell receptors (TSRs). They become effector cells (CD4+ helper T cells that promote the production of antibodies B-lymphocytes and phagocytosis, or CD8+ cytotoxic T cells that can lead to cell death) or memory cells [13]. Although an effective antitumor immune response involves a lot of components of the immune system, T-cells remain the most important cells that take part in the antitumour immunity [13]. Therefore, T-cell defects reduce the effectiveness of the antitumor immunity. These defects include: reducing of expression of CD3 – zeta chain (CD3z), key signaling molecule in TCR pathway [13], inability of T cells to destroy targets of tumor cells [14], a lack of IL-2 and / or IFN-γ [13], slowing down proliferative responses to mitogen or IL-2 [13], as well as the presence of pronounced apoptotic symptoms in a significant proportion of TIL (lymphocytes that infiltrate the tumor) [13].

A number of defects in TIL that are isolated from the tumor, have been identified in patients with oral cancer and oropharyngeal cancer. T-cell apoptosis was detected in a significant proportion of TIL [13], and was associated with Fas / FasL signaling [14]. The expression of FasL on the surface of tumor cells leads to an apoptotic signal in lymphocytes, causing a spontaneous loss of circulating Fas+ T-lymphocytes [8]. Other ways of TLI damage are associated with TNF-associated apoptotic-induced ligand (TRAIL), TNF-α antigen, FasL+, MHC Class I, and tumor derived membrane vesicles, all of which induce apoptosis in cells [8,13,25]

It is considered that the balance between subgroups of T-cells in cancer patients modulates antitumor immunity [9].

The effector cell of adaptive antitumor immunity is activated by CD8+ cytotoxic T-lymphocytes (CTLs). An activation of antigen-limited CD8+ cytotoxic T-lymphocyte initially requires binding of a T-cell receptor (TCR) to its corresponding TA in a complex with HLA-I. But this is not enough to activate cytotoxic T-lymphocytes and cytolysis of the tumor. The initial activation also depends on the balance of co-stimulating or co-inhibitory signals of dendritic cells and CD4+ T-helper

cells, as well as on avoiding CD4 suppression + regulatory T-cells (Treg). Cancer tumors induce anergy of T-cells in both peripheral and tumor-infiltrating lymphocytes (TILs). Functional defects in TIL affect the low productivity and response to IL-2 [5,13] and the susceptibility to spontaneous apoptosis mediated by the Fas / Fas-ligand by [8]. A low expression required for signaling TCR, co-stimulating molecules: CD3-ζ (part of the T-cell antigen receptor complex – TCR-CD3), OX40 (TNFRSF4 or CD134 – membrane protein, receptor from the superfamily receptor of the tumor necrosis factor of the ligand OX40L) and 4-1BB (CD137, TNFRSF9, a member of tumor necrosis factor superfamily 9) [18] and a high expression of co-inhibiting receptors – (CTLA-4) and a programmed protein cell death (PD-1) [4] cause changes in the regulation of immune responses. We should note that the PD-1, PD-L1 ligands are expressed in most tumor tissues of the oral cavity and oropharynx [9].

Cytotoxic T-lymphocytes are also inhibited by the disproportionate accumulation of Tregs (thymic regulatory T-lymphocytes) in the micro cavity of the oral cavity and oropharynx. Tregs promote signaling tolerance through an inhibitory CTLA-4 receptor [7]

CD4+ T-helper lymphocytes are the center of the antitumor response. CD4+ CD25+ T cells play a central role in initiating and maintaining antitumor immune response. Detecting of a large number of CD4 + CD25+ T- lymphocytes is associated with a good prognosis [17], although CD25+ is produced in small quantities by tumor cells. Also, the presence of CD4+ CD69+ T cells is associated with a good prognosis in patients with oral and oropharyngeal cancer [8].

The percentage of CD8+ T cells increased with the growth of the oral cavity and oropharynx, CD4+ T cells and Tregs in these patients are increased as well. The proportion of B-lymphocytes decreased in patients with local regional metastasis, while NK-cells decrease compared with the control group [6].

In patients with oral and oropharyngeal cancer, the following changes in the number of blood lymphocytes with markers of early and late activation were found: an increase in the percentage of CD25+ and the absolute number of CD 71+ lymphocytes, a slight increase in the number of CD16+ and a decrease in the relative number of cells ready for apoptosis-CD95+. Thus, the ratio of CD25 / CD95 and CD71 / CD95 cells is 2-2.5 times higher than in the control group, which indicates the predominance of positive lymphocyte activation processes in tumor development. It is also known that the percentage of CD3 is reduced, while CD8, CD22 increases without any changes in the absolute number of lymphocytes. This phenomenon is due to the migration and fixation of lymphocytes in the tumor [3].

Changes in the cellular composition of the immunity and effector line are observed in patients with cancer of the oral cavity and oropharynx. The number of activated T-cells with the CD45+ CD3+ HLA-DR phenotype is 2 times higher than in the control group, as well as the increased number of nonspecific effector cells with the CD45+ CD3+ CD5+, CD45+ CD8+ CD16+ phenotype.

Studying the immune status of patients with oral cancer and oropharyngeal cancer, a significant increase in the content of CD3+ T-cells with simultaneous expression of NK markers was found. The elevated blood levels of CD45+ CD3+ CD16+ CD56+ NK T-lymphocytes are determined 1.6 times, and CD45+ CD16+ NK cells are significantly elevated. Statistically significant increases in the overall level of CD45+ CD8+ lymphocytes with the expression of intracellular perforin (CD45+ CD8+ Perforin+) and NK cells with the CD45+ CD16+ Perforin+ phenotype, which is the evidence of increased activity of the effector immunity. Indicators of the latest increased due to the subpopulation of nonspecific effector cells with the phenotype CD45+ CD3- CD8+. And the number of CD45+ CD3+ CD4+ T-lymphocytes is reduced.

The study of CD45+ CD3- CD19+ B-lymphocytes in patients with oral cancer and oropharyngeal cancer are not revealed a statistical difference from the control group [3, 6].

In addition, tumor cells can avoid the recognition of T-lymphocytes by reducing the transporter associated with the heterodimer of antigen processing (TAP-1/2). This process is controlled by IFN-γ phosphorylated signals and transcription activators of the mediated signaling path (pSTAT1) [16].

Tregs and their role in the immune response in patients with oral and oropharyngeal cancer.

Tregs are tumorous regulatory T-lymphocytes, a subpopulation of suppressor T-cells that respond to the inflammation. They are involved in the construction of immune responses and according to research data that revealed an excessive expression of Tregs, they are inhibitors of antitumor immune responses [9], that is, they contribute to the evasion of tumor cells from the immune response and the progression of oral cancer and oropharyngeal cancer [8, 17]. Tregs also play a major role in maintaining the tolerance of T-cells to the antigens themselves [8]. The antitumor immune response is suppressed by Tregs subtype Tr1 in the microenvironment of the tumor.

Various factors can enhance the production of Tregs in the microenvironment of the tumor. An overexpression of cyclooxygenase 2 (COX-2) and PGE2 synthesis in patients with oral cancer and oropharyngeal cells induce the generation of Tregs type1 in the microenvironment of the tumor, which enhance its carcinogenicity and progression of the disease [6, 8]. Particularly important are Tregs type 1 cells with a phenotype other than CD4+ CD25 (high) FoxP3+ Tregs. Tr1 produces IL-10 and TGF-β1. They mediate IL-10-dependent immune oppression in cells by contact-independent method [8]. IL-10 itself induces Tregs [21].

Another factor influencing the production of Tregs is HMGB1-chemoattractant from a group of non-gistonal B1 proteins, the levels of which in serum are significantly elevated in patients with oral and oropharyngeal cancer. And a chemokine like CCL22 is an intermediary in the migration of Tregs to the tumor site. Its corresponding receptor, CCR4, is elevated in patients with oral and oropharyngeal cancer [17].

The enhanced Tregs tumor infiltration promotes the expression of HMGB1 recognition receptor toll-like receptor 4 (TLR4) [24]. Tregs produce a lot of TLRs, because they play an important role in identifying molecules that are different from host molecules. A high expression of these receptors - TLR4, TLR6, TLR9 and TLR10 - was detected in patients with oral and oropharyngeal. Linking TLR to Tregs increases their suppressive activity, which promotes tumor-mediated immune suppression [23]. It has been determined that Tregs suppressive function is significantly higher with HSP60 or lipopolysaccharides.

In several studies of patients after the resection of the oral cavity and oropharyngeal tumors, in which there was no progression or relapse over the years, an increased number of identified Tregs was detected and that was not associated with the tumor stage. This shows that an oncotherapy contributes to the growth and spread of Tregs [21].

The intranuclear regulatory factor – FoxP3 controls regulatory activity Tregs. Tumor cells synthesize it themselves, so it is an independent prognostic indicator of the squamous cell carcinoma of the tongue. Its expression is associated with the differentiation and stage of cancer and is inversely proportional to survival rates [13].

An increased number of Tregs in the lymph nodes has already been observed in the presence of a dysplastic state or early stage of oral and oropharyngeal cancer. And their increase is accompanied by the growth of different populations of immune cells that exhibit a positive antitumor response. These cells include conventional T-cells (Tconv) – CD4 T-lymphocytes, T-helper 1 (Th1) and CD8+ T lymphocyte [12]. The total number of lymphocytes in patients with oral and oropharyngeal cancer is lower and on this background there is a decrease in the proportion of CD4+ T cells, CD8+ T cells, CD3- CD56+ CD16+ NK cells and CD3+ CD56+ NKT cells. However, the proportion of CD4+ CD25+ FoxP3+ Tregs with suppressor functions is still increasing [6].

The role of NK-cells in antitumor immunity in patients with oral and oral cancer.

NK-cells are the major cells of innate immunity, large granular lymphocytes and play a decisive role in the antitumor activity. They are capable of recognizing and destroying transformed malignant cells without a specialized MHC-antigen presentation. In particular, they carry out antibody-dependent cell-mediated cytotoxicity (ADCC). Their varieties - CD1d-restricted NKT cells (iNKT) play an important role in the activation of immune effector cells. It has been established that in patients with oral and oral cancers their number is significantly reduced, respectively, the low control of iNKT tumor cells in the regional collector, which correlates with low survival rates of these patients [17]. Functioning of NK cells is important for the cytokine environment, since activated NK cells secrete immunostimulating cytokines IFN-γ and TNF-α, which have their antitumor function.

Among the lymphocyte populations there is an increase in the relative and absolute number of innate immune cells - NK cells. NK cells occupy a central place in antitumor immunity and control the growth of the tumor at all stages (including metastasis) [3]. However, in some other studies, depressed NK-cytotoxic function is observed. It is believed to be associated with a decrease in the expression of cytotoxic molecules such as perforin, granzyme B and FasL in mononuclear cells of peripheral blood of these patients [6].

Tumor cells of the oral cavity and oropharynx produce a large amount of TGF-β1 that reduces the expression of NK-cell receptors (NKG2D is the primary cytotoxicity receptor), and CD16 (or Fcγ-receptor (Fcγ-R) III), suppressing the biological functions of NK cells [13]. The Fcγ receptor (Fcγ-R) III is a mediator of antibody-dependent cell-mediated cytotoxicity (ADCC).

Influence of macrophages on the development of oral and oropharyngeal cancer.

Macrophages are involved in both natural and acquired reactions of the immune system. These include induction of immunity, antigen presentation, cell cytotoxicity, tissue remodeling, inflammation regulation, thrombosis and endocytosis [10,13,25]. It has been established that macrophages contribute to the neovascularization, which is a compulsory requirement for the growth and spread of the tumor, that is, they have swollen functions. It has been shown that VEGF, a specific endothelial cell growth factor, is secreted by macrophages in patients with different carcinomas [13]. Thus, in patients with oral and oropharyngeal cancers, it has been proved that an increase in the number of tumor associated macrophages (TAMs) is a sign of tumor invasion, a greater intramatural density of micro vessels in the tumor and expression of VEGF [15] in patients with oral and oropharyngeal cancer, as well as more rapid tumor progression [13].TABs have been identified in fibrin deposition areas, indicating that they play an important role in stabilizing intramatural fibrin, as well as in promoting tumor matrix generation [11]. Tumor cells of the oral cavity and oropharynx can involve macrophages through secretion of monocytic chemotactic protein-1 (MCP-1) and TGF-β1. Then the macrophages secrete VEGF and IL-8, TNF-α and IL-1, which stimulate the release of tumor cells more than VEGF and IL-8 [13].

An interaction of systemic and local immunity in patients with oral and oropharyngeal cancer.

The interaction of systemic and mucosal immunities is important in protecting the mucous membrane from the action of the nonplastic process. There is a decrease in the total number of segmental neutrophils, monocytes, platelets in the blood of patients with oral and oropharyngeal cancer. Apoptosis indicators of lymphocytes are significantly lowered, which is considered a sign of the immune response to the tumor development, aimed at preserving and increasing the total number of lymphocytes [13].

Lactoferrin is involved in the antitumor protection of patients with oral and oropharynx cancer, but its amount in the peripheral blood does not change, which is the result of a decrease in the circulation of the producing cells of this protein (segmental proteins) [3].

The immunodeficiency of patients with oral and oropharyngeal cancer is shown by elevated indicators of circulating immune complexes (CICs). Also, it was retrospectively found that in patients with a favorable prognosis of the treatment is 2 times lower compared with patients who subsequently had a relapse [3].

In the medical literature there is an evidence that epithelial cells of the tumor of the oral cavity and the oropharynx are capable of producing nitric oxide and its derivatives. Analyzing the nitroxydergic regulatory system of blood in these patients, there was detected an increase in the index of end metabolites of NO - NO3 in 1.4 times. It somewhat affects the IFN-γ and antibodies to it, however, the way of its affecting is still being investigated.

Changes in TNF-α and IFN-γ on the salivatory level are also not detected, but the antibody level decreases to IFF-α in several times the, which has a negative effect on the local antitumor protection [3].

When comparing the state of systemic and mucosal immunity in patients with oral and oropharyngeal cancer before and after chemoradiotherapy, it has been found that after the treatment there is an enhanced synthesis of such cytokines as TNF-α in blood, TNF-α and INF-γ in saliva, and an excessive synthesis of terminal stable metabolites NO. The chemoradiotherapy has a negative effect on the systemic immunity. It causes the development of leukopenia, lymphopenia, a decrease in the number of major populations of lymphocytes - NK, CD3, CD4, CD8, CD22, and the number of cells with positive activation markers – CD25+, CD71+, HLA-DR+ [3].

Mucosal (local) immunity of patients with oral and oropharyngeal cancer.

An increase in the indicator of the state of innate immunity - mucin - is shown in the saliva, because it is further synthesized directly by epithelial malignant cells. Mucin has an immunosuppressive effect, that is, it promotes the anergy of T-lymphocytes, the induction of tolerance of cytotoxic lymphocytes, it also helps the antigen to avoid the immune response and reduces the synthesis of some cytokines [3].

An increased activity of the end component of complement C5 in saliva is determined due to the absence of its fixation in the membrane-attacking complex in the target cells, which is a cancerous cell. This can affect the formation of the immune response, the processes of the migration of macrophages and leukocytes at the site of development of the tumor.

In patients with oral and oropharynx due to the fixation of some subclasses of immunoglobulins on the surface of the tu-

mor, as well as a decrease in their synthesis by mucosal-associated lymphoid tissue in saliva, the reduction of the subclass of IgG4 is shown without altering the general population [3].

sIgA is an indicator of a local immunological response. IgA is synthesized locally by plasmatic cells in the interstitial tissue between the acinus of the salivary glands and in the subepithelial layer of the mucous membrane of the mouth. Then the IgA molecule forms a complex with a secretory component in the glandular epithelium – sIgA. In healthy people, the concentration of sIgA in saliva is 0.03-0.26 g/l. These numbers are lower in chronic smokers – 0,01-0,05 g/l, and in patients with carcinoma of the oral cavity and oropharynx, it is reduced by 45% [13].

Other salivary components of patients with oral cancer and oropharyngeal were evaluated: Amylase is 25% lower, IgG is 125%, albumin is higher than 108%, lactate dehydrogenase – 88%, insulin growth factor – 117%. Also, MMP-2 and MMP-9 are higher respectively by 75% and 35%. The indicator of the epidermal growth factor remains unchanged. There are also some changes in the electrolyte balance of saliva: K rates decrease by 155, Na – by 14%, Ca by 59%, P by 39%, Mg by 28%.

The mucous membrane of the oral cavity and oropharynx by enzymatic cleavage of the damaging agent, phagocytic reaction, the synthesis of antimicrobial substances fulfills its barrier function and provides natural immunity. APK (macrophages, dendritic cells, intergranular cells), some subpopulations of T lymphocytes, and polymorph nuclear neutrophils are cellular elements of nonspecific oral and oral defense that are responsible for the condition of local immunity. A study of functional activity of cells of granulocyte-macrophage (non-specific) links of immunity of patients with oral and oropharyngeal cancer was conducted. The bactericidal and fungicidal function of monocytes are significantly lowered. The relative number of polymorph nuclear leukocytes with phagocytic activity is lower compared with healthy ones, and the number of phagocytic monocytes does not change [3].

Conclusions.

1. There is an imbalance in the immune system in patients with oral and oropharyngeal cancer, but more specifically its role in initiating and progressing cancer of the oral cavity and oropharynx is only now, after a modern research becomes more understandable.
2. Mutated, atypical cells are eliminated by the immune system rather than they form a tumor as it is immune control, and the violation in it contributes to the immune avoidance of atypical cells, which can lead to cancer.
3. Tumor cells use the immune system in several ways to stimulate angiogenesis, proliferative signals and, in general, tumor progression. It can mask itself from the immune system by self-modification and immunosuppression of the patient's body.
4. Tumor synthesizes: cytokines, (TGF-β), interleukins (IL-6, IL-10), inflammatory factors of transcription (NF-kB), STAT3, suppressing cellular antitumor immunity.
5. A deep understanding of antitumor immune effects in cancer patients and their associated mechanisms for avoiding an immune response by a tumor cell prompts the search and usage of more targeted cancer therapy - immunotherapy in combination with standard methods.

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SUMMARY

ANALYSIS OF IMMUNE CHANGES AND THEIR ROLE IN THE DEVELOPMENT OF ORAL AND OROPHARYNGEAL CANCER

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Numerous studies of the immune system in cancer patients at the cellular and molecular levels indicate a persistent violation of natural and acquired mechanisms of immune defense.

This article reveals changes in the immune system of patients with cancer of the oral cavity and oropharynx. It was determined that the levels of cytokines IL-6, IL-10, HGF and VEGF in high concentrations in the serum of patients correlate with an unsatisfactory prognosis and are independent indicators of low survival and correlate with relapse. Different immune cells provide a complex defense system with effective communication through cytokines. The inflammatory component of the microenvironment of the tumor of the oral cavity and oropharynx is important in the immune response, because its main component is immunosuppressive cytokines. There is an imbalance of Th1 / Th2 cytokine types and increased levels of Tregs. Among Th1 types of cytokines - IFN-γ, IL-12, TNF-α, the levels of which are reduced, and Th2-cytokines - IL-4, IL-10 - are increased, while the norm is the opposite.

The tumor uses various mechanisms to induce changes in the system to avoid an immune response to its development. In fact, a malignant cell of the mouth or oropharynx suppresses the immune response by producing vascular endothelial growth factor (VEGF), prostaglandin E2 (PGE2), TGFβ, IL-6 and IL-10. The tumor environment also releases pro-inflammatory mediators, including a receptor such as IL-15 alpha subunit (IL15RA). It in combination with IL-15 carries out the strengthened synthesis of proinflammatory cytokines - IL-6, TNF-α and IL-17 that influences insufficiency of the immune response and accordingly low prognosis of survival.

It was found that the number of NK cells is reduced in patients with oral and oropharyngeal cancer, respectively, there is a low control of iNKT tumor cells in the regional collector, which correlates with low survival rates.

Increased concentration of dendritic cells in the blood correlates with a positive prognosis and greater survival of patients with cancer of the oral cavity and oropharynx, reduced metastasis to the cervical lymph nodes.

An effective antitumor immune response involves many components of the immune system, but T-cells remain the most important cells involved in antitumor immunity. Therefore, T-cell defects reduce the effectiveness of antitumor immunity. CD4 + CD25 + T-cells play a central role in initiating and maintaining the antitumor immune response. Detection of them and CD4 + CD69 +, CD3 + T cells in large numbers is associated with a good prognosis. More detailed changes in the cellular composition of immunity and the effector link are presented in the article.

Keywords: cancer, oral cavity, oropharynx, antitumor immunity.

РЕЗЮМЕ

АНАЛИЗ ИММУНОЛОГИЧЕСКИХ ИЗМЕНЕНИЙ И ИХ РОЛЬ В РАЗВИТИИ РАКА ПОЛОСТИ РТА И РОТОГЛОТКИ (ОБЗОР)

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Многочисленные исследования состояния иммунной системы у онкостоматологических больных на клеточном и молекулярном уровнях свидетельствуют о стойком нарушении природных и приобретенных механизмов иммунной защиты.

В статье раскрыты изменения в иммунной системе больных раком полости рта и ротоглотки. Определено, что уровни цитокинов IL-6, IL-10, HGF и VEGF в высокой концентрации в сыворотке крови больных указывают на неудовлетворительный прогноз лечения, являются показателями низкой выживаемости и коррелируют с рецидивом. Клетки иммунитета обеспечивают сложную систему защиты через цитокины. Воспалительный компонент микросреды опухоли полости рта и ротоглотки играет значимую роль в иммунном ответе, поскольку основным его компонентом являются иммунодепрессивные цитокины. Уровни Th1 цитокинов - IFN-γ, IL-12, TNF-α понижены, а Th2-цитокины - IL-4, IL-10 - повышены, тогда как в норме показатели противоположны.

Опухоль использует различные механизмы для индукции изменений в системе во избежание иммунного ответа на ее развитие, в частности злокачественная клетка полости рта или ротоглотки путем выработки фактора роста эндотелия сосудов (VEGF), простагландина E2 (PGE2), TGFβ, IL-6 и IL-10 подавляет иммунный ответ. Опухолевая среда высвобождает провоспалительные медиаторы, в том числе такой рецептор как IL-15 alpha subunit (IL15RA), который в сочетании с IL-15 осуществляет усиленный синтез провоспалительных цитокинов - IL-6, TNF-α и IL-17, влияет на недостаточность иммунного ответа и соответственно низкий прогноз выживаемости.

Установлено, что у больных раком полости рта и ротоглотки количество NK-клеток понижено, соответственно возникает низкий контроль iNKT опухолевых клеток в регионарном коллекторе, что коррелирует с низкими показателями выживаемости.

Повышенная концентрация дендритных клеток в крови коррелирует с позитивным прогнозом и большой выживаемостью больных раком полости рта и ротоглотки, уменьшением метастазирования в шейные лимфатические узлы.

Эффективный противоопухолевый иммунный ответ включает множество компонентов иммунной системы, однако Т-клетки остаются самыми значимыми клетками, участвующими в противоопухолевом иммунитете. Поэтому Т-клеточные дефекты снижают эффективность противоопухолевого иммунитета. CD4 + CD25 + Т-клетки играют центральную роль в инициировании и поддержке иммунного ответа. Наличие в большом количестве вышеуказанных и CD4 + CD69 +, CD3 + Т-клеток связано с хорошим прогнозом.

რეზიუმე

იმუნოლოგიური ცვლილებების ანალიზი და მათი როლი პირის ღრუს და პირხახის კიბოს განვითარებაში

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ონკოსტომატოლოგიური ავადმყოფების იმუნური სისტემის მდგომარეობის უჯრედულ და მოლეკულურ დონეზე ჩატარებული მრავალი კვლევა მიუთითებს იმუნური დაცვის ბუნებრივი და შეძენილი მექანიზმების მდგრად დარღვევაზე.

სტატიაში აღწერილია პირის ღრუს და პირხახის კიბოთი პაციენტების იმუნური სისტემის ცვლილებები. დადგინილია, რომ მაღალი დონის კონცენტრაციის ციტოკინების IL-6, IL-10, HGF და VEGF არსებობა პაციენტთა სისხლის შრატში მიუთითებს მკურნალობის არადამაკმაყოფილებელ პროგნოზზე, წარმოადგენს დაბალი გადარჩენის მაჩვენებელს და კორელაციაშია რეციდივთან. იმუნური უჯრედები უზრუნველყოფენ ციტოკინების მეშვეობით დაცვის კომპლექსურ სისტემას. პირის ღრუს და პირხახის სიმსივნის მიკროგარემოს ანთებითი კომპონენტი მნიშვნელოვანია იმუნური რეაქციის დროს, ვინაიდან მის ძირითად შემადგენელს წარმოადგენს იმუნოსუპრესიული ციტოკინები. Th1 ტიპის ციტოკინებს შორისაა IFN-γ, IL-12, TNF-α, რომელთა დონე დაქვეითებულია და Th2-ციტოკინები - IL-4, IL-10, რომელთა დონე მომატებულია, ხოლო ნორმაში ეს მაჩვენებლები საპირისპიროა.

პირის ღრუს ან პირხახის ავთვისებიანი უჯრედი თრგუნავს იმუნურ პასუხს სისხლძარღვთა ენდოთელიუმის ზრდის ფაქტორის (VEGF), პროსტაგლანდინის E2 (PGE2), TGFβ, IL-6 და IL-10 წარმოქმნით. სიმსივნის გარემო გამოყოფს ანთებისსაწინააღმდეგო მედიატორებს, მათ შორის ისეთ რეცეპტორს, როგორიცაა IL-15 alpha subunit (IL15RA), რომელიც IL-15- თან ერთად ახორციელებს ანთებისსაწინააღმდეგო ციტოკინების - IL-6, TNF-α და IL-17 გაძლიერებულ სინთეზს, გავლენას ახდენს იმუნური პასუხის დეფიციტზე და, შესაბამისად, გადარჩენადობის დაბალ პროგნოზზე.

აღმოჩნდა, რომ პირის ღრუს და პირხახის კიბოთი დაავადებულ პაციენტებში NK უჯრედების რაოდენობა შემცირებულია; შესაბამისად, რეგიონულ კოლექტორში ვლინდება სიმსივნის უჯრედების დაბალი iNKT კონტროლი, რაც კორელაციაშია გადარჩენადობის დაბალ მაჩვენებლებთან.

დენდრიტული უჯრედების მომატებული კონცენტრაცია სისხლში კავშირშია პირის ღრუს და პირხახის კიბოს მქონე პაციენტების დადებით პროგნოზთან და გადარჩენის მაღალ მაჩვენებელთან და კისრის ლიმფურ კვანძებში მეტასტაზირების შემცირებასთან.

ეფექტური სიმსივნისსაწინააღმდეგო იმუნური პასუხი მოიცავს იმუნური სისტემის მრავალ კომპონენტს, თუმცა Т-უჯრედები რჩებიან უმნიშვნელოვანეს უჯრედებად, რომლებიც მონაწილეობენ სიმსივნის საწინააღმდეგო იმუნიტეტში. ამიტომ, Т უჯრედების დეფექტები ამცირებს სიმსივნისსაწინააღმდეგო იმუნიტეტის ეფექტურობას. CD4 + CD25 + Т უჯრედები ცენტრალურ როლს ასრულებენ იმუნური პასუხის ინიცირებასა და შენარჩუნებაში. მათი და დიდი რაოდენობით CD4 + CD69 +, CD3 + Т- უჯრედების გამოვლენა კარგ პროგნოზს უკავშირდება.

PREVALENCE AND RISK-FACTORS OF BRUXISM IN CHILDREN AND ADOLESCENT POPULATION AND ITS IMPACT ON QUALITY OF LIFE (REVIEW)

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Bruxism is a disorder that is characterized by grinding and clenching the teeth either in sleep or also awake. It has become an increasing problem all over the world, considering its negative impact on the quality of life in children and adult population. Etiology of bruxism is multifactorial, but to summarize it can be divided into two factors: peripheral (morphological) and central (pathophysiological, psychological). Peripheral plays a minor role and major regulator of bruxism is central. The knowledge and approach to understanding the pathology is changing as new definitions, classifications and theories regarding etiology has been accumulated with the evolution and growth of knowledge of this subject [13]. However, last studies define that bruxism is a psychophysiological disorder [18]. In addition, bruxism is no longer accepted as a single entity and it has two distinct circadian manifestations. These 2 distinct manifestations of bruxism may take place in different circadian phases, creating sleep and/or awake bruxism, which may share common risk factors and lead to similar consequences on the masticatory system, although etiology and pathophysiology may differ [1]. The risk factors and associated problems of bruxism are amply documented and studied, however the recent publications point out that personality features, such as anxiety traits and stress sensitivity, are the main psychological factors associated with bruxism, both in children/adolescents and in adults [25]. As a result, bruxism may lead to the health problems such as tooth wear, tooth mobility, and other clinical findings like tongue/cheek indentation, masticatory muscle hypertrophy, temporomandibular joint pain, headaches, and masticatory muscle pain or fatigue [15]. Due to unfavorable effects that sleep or awake bruxism has on the oral hard and soft tissue, the dental health care practitioners are particularly concerned with it [22]. Therefore, a sound knowledge of the physiopathology of this parafunction together with the etiologic and associated factors is needed for properly screening the various forms of bruxism in children and adolescents [24].

This is a review paper and hence study discusses the possible risk-factors leading to bruxism in children and adolescent population. Moreover, the paper looks into its pathological impact affecting individual's health and quality of life as well as the methods of treatment. The focus group of this review is children and adolescents, because bruxism is observed to be more prevalent in these groups.

The review of the literature has been carried out using the "ScienceDirect", "Scopus" and "PubMed" scientific bases in order to define relevant scientific works - published in English, not earlier than 2014. Following keywords are used: bruxism, tooth wear, sleep apnea, sleep and awake bruxism, bruxism prevalence. Over 200 articles were analyzed and 31 most relevant articles were chosen and analyzed in details.

Classification of Bruxism. Bruxism is classified according to: presence, occurrence, etiology, and motor activity [29]. The presence of bruxism can be the past bruxism, which is currently non-active and the present bruxism, which is currently active [29,31]. Three types of bruxism can be identified according to its occurrence: sleep bruxism, which occurs when individual sleeps, awake bruxism which occurs while individual is awake and combined bruxism, which occurs in both situations [31]. Etiologically the bruxism can be primary and secondary. In the case of primary bruxism the cause factors are not identifiable. However, in the case of secondary bruxism, causing factors can be presented as a consequence of neurologic, psychiatric, sleep or movement disorders, or of an iatrogenic type associated with drug use/withdrawal [31]. Motor activity type of bruxism can be classified into three groups: tonic, phasic and combined. A tonic bruxism is when the muscular contraction lasts less than 2 seconds, while the phasic bruxism is characterized by the brief repeated muscular contractions with at least three consecutive electromyographic bursts of 0.25 and 2 seconds. A variation of tonic and phasic episodes is the combined bruxism [29].

Risk-factors of Bruxism. Commonly, the risk factors of bruxism are known to be: gender, age, genetics, endocrine disorders, allergies, enlargement of the tonsils, cheeks tonus, perioral musculature participation, biting, mouth breathing (day and/or night), headaches, mental health problem, anxiety, emotional instability, psychological factors, hyperactivity, medication, noisy bedroom and even family income [2,8,9,11,14]. Pediatric dentistry classifies 3 groups of causing factors:

1. Local factors including occlusal interferences, high or poor filling restorations.
2. Systematic factors including malnutrition and nutritional deficiencies, allergies, endocrine disorders;
3. Psychological factors including personality disorders and increased stress [11].

However, several risk factors for bruxism in children and adolescents are identified among which behavioral abnormalities and sleep disturbances predominated [14]. A statistically significant association was found between childhood bruxism and restless sleep in children. Children with restless sleep are more likely to exhibit bruxism [20]. Mental disorders, mainly anxiety disorders, and other psychological variables were also significantly related to tooth grinding during sleep. Based on the available evidence, a significant association between sleep bruxism and stressful, anxious, and tense personality traits is found in children aged between 6 and 11 years; likewise, a significant association between sleep bruxism and psychosocial disorders was present in adolescents of age between twelve and seventeen years old [30]. In a group of adolescents legal psychoactive substances intake showed moderate association with sleep bruxism [3].

Prevalence of Bruxism. A cross-sectional study of 151 preschool children revealed the prevalence of bruxism to be 45.0% associated with the location of headache as well as some parafunctional habits [6]. Correspondingly, another school-based cross sectional study of total 935 children aged between 2-5 and 8-10 years demonstrated that the prevalence of bruxism was 22.3% in 2-5 age group and 32.7% in 8-10 age group related with poor sleep quality [17]. Furthermore, the clinical examination of 253 undergraduate students was in concordance with the numbers of previous research, bruxism occurred in 31.6% most associated with bruxism were stress, muscle pain, TMJ pain, and TMJ noise [26].

Effects of bruxism on the health. Statistically significant data proves association between oral health and general health

[16,21,27]. Bruxism can cause short-term and long-term, permanent effects on individual's health conditions. Short-term effects of bruxism include: tooth sensitivities,headaches, facial myalgia, ear ache, tightness/stiffness of the shoulders, limitation of mouth opening, sleep disruption, sleep disruption of bed partner due to noise, excess tooth mobility, inflamed and receding gums. While long-term effects are Temporomandibular Joint Disorder, tooth wear and breakage, tongue's deformation [5,10,11].

The reviewed literature and statistically significant data prove that bruxism still remains a serious problem all over the world. The findings demonstrate many different factors associated with jaw clenching and tooth grinding. Recognition of the possible causes, clinical characteristics, signs and symptoms of the bruxism in childhood and elimination of the problem as early as possible is very important in order to avoid further difficulties [20]. According to literature, it is evident that there are not any general medical procedures for treating bruxism and each individual case requires specific way of treatment. The aim of treatment is to find and remove the factors causing bruxism, change the behavior and eventually repair the caused harm.

The dentists usually act through restorative procedures and occlusal splints, but in some specific cases, systemic treatment using pharmacological prescriptions is needed, associated with medical and psychological support [4].

Methods of treatments preferable for sleep bruxism includes: occlusal therapy, pharmacological therapy, contingent electrical stimulation, behavioral treatments and biofeedback [9]. Improving the quality of sleep, reducing the use of stimulants such as caffeine and nicotine, having a good bedtime routine and relaxing before bed are some of them [12]. On the one hand interventions such as counselling about triggers, habits modification, relaxation therapy, or biofeedback treatment methods are appropriate for treating of awake bruxism [7].

Considering the potential negative consequences of persistent bruxism on dental and oral health, the following recommendations can be given: self-observation for bringing awareness of clenching or grinding activities during waking hours; muscle relaxation, for a "muscular and vegetative stabilization" In order to achieve an improved body perception and stress management; and splint therapy, to protect the tooth structures from attrition other oral structures from overload, and also to protectt possible dental reconstructions from damage [14].

Conclusion. Bruxism is a common disorder among children and adults, becoming a growing concern for both family members and health professionals. As it was mentioned above, bruxism is a complex disorder with a controversial etiology [19]. It is obvious that this parafunctional habit whether sleep and/or awake, consists in an important change in the oral sensory-motor system, which requires a multidisciplinary approach, in order to reduce injuries in dentognathic structures [28]. In this context studies suggest a variety of treatment methodologies including dentistry, physiotherapy and psychology.

Despite the fact that several psychoactive drugs and behavioral therapies are used as a treatment methodologies, they are still effective only for limited samples and with the risk of severe side-effects. Thus, the further research, treatment strategies as well as outreach is needed, to show how widespread the disease is, how severely it may affect the helath and promote the actions to decrease the prevalence of bruxism, particularly in children and young adults.

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SUMMARY

PREVALENCE AND RISK-FACTORS OF BRUXISM IN CHILDREN AND ADOLESCENT POPULATION AND ITS IMPACT ON QUEALITY OF LIFE (REVIEW)

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Bruxism has become more and more debatable and pressing issue all over the world last years. the etiology of bruxism has been changing diverse definitions, over the years, however recently it is defined as a repetitive jaw-muscle activity characterized by clenching or grinding of the teeth and by bracing or thrusting of the mandible. This literature review discusses the possible risk factors of bruxism in children and adolescence, among which behavioral abnormalities and sleep disturbances predominates. Moreover, it reviews pathological impact of bruxism on general health and quality of life.

The literature review has been carried out using the "ScienceDirect", "Scopus" and "PubMed" databases in order to define relevant scientific works - published in English, during the last 5 years. 31 most relevant articles were chosen.

Bruxism is a psychophysiological disorder that can take place during the day and/ or night, in a form of clenching and grinding. It can cause health problems such as tooth sensitivities, headaches, facial myalgia, ear ache, tightness/stiffness of the shoulders, limitation of mouth opening, sleep disruption, sleep disruption of bed partner due to noise, excess tooth mobility, inflamed & receding gums, Temporomandibular Joint Disorder, tooth wear and breakage and tongue's deformation. Considering the potential negative consequences of bruxism on dental and oral health, various clinical methods have been devised to assess it over the years. As the etiology is multifactorial, there is no exact treatment to prevent bruxism. Counselling and behavioral strategies, splint therapy, medications, and contingent electrical stimulation can be used as different ways reducing the effects of bruxism

Keywords: bruxism, tooth wear, sleep apnea, sleep and awake bruxism, bruxism prevalence.

РЕЗЮМЕ

ПРЕВАЛЕНТНОСТЬ И ОПРЕДЕЛЕНИЕ РИСК-ФАКТОРОВ БРУКСИЗМА В ПОПУЛЯЦИИ ДЕТЕЙ И ПОДРОСТКОВ И ЕГО ВЛИЯНИЕ НА КАЧЕСТВО ЖИЗНИ (ОБЗОР)

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В последние годы бруксизм является актуальной и спорной темой во всем мире. Со временем меняется как его вызывающие причины, так и классификация и методы лечения. В обзоре обсуждаются возможные риск-факторы развития бруксизма в детском и в взрослом возрасте, его патологические результаты, влияющие на здоровье и качество жизни человека.

Литературный обзор основан на данных «ScienceDirect», «Scopus» и «PubMed» за последние 5 лет. Отобрана 31 научная релевантная статья на английском языке.

Бруксизм – психофизиологическое нарушение, которое проявляется скрипом зубов, вызывая целый ряд неудобств: боль в области лица, чувство напряжения в области плеч, неполноценный сон, нарушение функционирования височно-нижнечелюстных суставов, стираемость зубов, воспаление десен, боль в области уха. Данные симптомы отрицательно влияют на качество жизни человека.

На сегодняшний день разработано множество методов лечения бруксизма, однако ввиду разнообразия вызывающих бруксизм факторов какого-либо уникального метода его лечения не имеется. Методами лечения бруксизма являются медикаментозное лечение, электростимуляция и сплинт-терапия. Результаты проведенных аналитических работ диктуют необходимость продолжения исследований по разработке уникального, всестороннего и полноценного метода лечения бруксизма.

რეზიუმე

ბრუქსიზმის პრევალენტობისა და რისკ-ფაქტორების განსაზღვრა ბავშვთა და მოზარდთა პოპულაციაში და მისი გავლენა ცხოვრების ხარისხზე (მიმოხილვა)

თ.წითაძე, ს.ფუთურიძე, თ.ლომთაძე, ვ.მარგველაშვილი, მ.კალანდაძე

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ბოლო წლების განმავლობაში ბრუქსიზმი მეტად აქტუალური და საკამათო თემა გახდა მთელი მსოფლიო მასშტაბით. დროთა განმავლობაში იცვლება, როგორც მისი გამომწვევი მიზეზები, ასევე კლასიფიკაციები და მკურნალობის მეთოდები. მიმოხილვაში განხილულია ყველა შესაძლო რისკ ფაქტორები, რომლებიც კავშირშია ბრუქსიზმის განვითარებასთან ბავშვებსა და მოზარდებში, ასევე, მათი ზეგავლენა ადამიანის ჯანმრთელობასა და ცხოვრების ხარისხზე.

ლიტერატურული მიმოხილვა ეფუძნება "Science Direct", "Scopus" და "PubMed" მონაცემთა ბაზებს, კერძოდ სამეცნიერო ნაშრომებს ინგლისურ ენაზე, და გამოქვეყნებულია ბოლო 5 წლის განმავლობაში. განხილულია 31 სტატია.

ბრუქსიზმი არის ფსიქოფიზიოლოგიური დარღვევა, რომელიც გამოიხატება დღისით ან და ღამით კბილების კრაჭუნით, იწვევს მთელ რიგ პრობლემებს: ტკივილს სახის და მხრების არეში დაჭიმულობის შეგრძნებას, არასრულფასოვან ძილს, კბილების მაგარი ქსოვილის ცვეთას, კბილების მორყევას, საფეთქელ-ქვედა ყბის სახსრის ფუნქციონირების დარღვევას, ღრძილების ანთებას, ტკივილს საფეთქლის არეში, თავის ტკივილს, რაც უარყოფიდად მოქმედებს ადამიანის ცხოვრების ხარისხზე.

სადღეისოდ შემუშავებულია ბრუქსიზმის სამკურნალო უამრავი მეთოდი. გამომწვევი მიზეზების მრავალფეროვნების გამო არ არსებობს რაიმე უნიკალური მეთოდი მის სამკურნალოდ. ექიმთან კონსულტაციები, მედიკამენტებისა და ელექტრო სტიმულაციის გამოყენება და სპლინტ-თერაპია წარმოადგენს სხვადასხვა გზას ბრუქსიზმთან საბრძოლველად.

COMPARATIVE EVALUATION OF THE CLINICAL EFFICACY OF MODERN REMINERALIZING DRUGS IN THE TREATMENT OF ENAMEL CARIES (FOCAL DEMINERALIZATION)

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Tooth caries is a multifactorial disease, in the development of which enamel resistance plays an important role [4,25]. It is known that the development of caries is due to the demineralization of tooth enamel with acids, which are the product of fermentation of dietary carbohydrates by bacteria of dental plaque [28]. Caries is a dynamic and reversible process; an understanding of this fact has led to the development of new technologies that can diagnose caries at the earliest stages for its timely treatment and prevention [5,22]. The clinical efficacy of fluoride in the prevention of caries has been proven by numerous studies [1,6]. As remineralizing agents, agents containing various fluorine compounds are used: sodium fluoride, aminofluoride, sodium monofluorophosphate, etc [8]

In the 1970s, Professor A. Knappvost developed a method for deep fluoridation of hard dental tissues. Deep fluoridation is based on chemical reactions that occur during the sequential processing of hard tooth tissues with a solution of magnesium and copper fluoride silicates and a suspension of highly dispersed calcium hydroxide, which leads to the formation of a fluorosilicate complex [7]. The complex spontaneously disintegrates with the formation of microcrystals of calcium fluoride, magnesium, copper, and polymerized silicic acid. Fluoride crystals are located on the surface and deep inside the enamel in a thixotropic silica gel, due to which there is a prolonged release of fluoride ions in a concentration sufficient for remineralization [13,24].

It is known that fluorides remain the leading means in the prevention and treatment of initial lesions of hard dental tissues [19]. The enamel remineralization in this case is due to the formation of a more durable fluorapatite based on epitaxial growth of residual crystals. However, the formed crystals of apatite may differ in their physicochemical and strength properties from intact enamel crystals [10,17]. Since mature enamel is a dead tissue, almost devoid of proteins involved in regulating the processes of building hydroxyapatite crystals, an ideal remineralization drug should ensure the organization and formation of micro-architecture of hydroxyapatite crystals, which are most similar to those of healthy enamel [12,23,27]. Currently, a drug for biomimetic recovery of enamel based on amelogenin protein "InnoDent" (PLC, "InnoDent", Kazakhstan) has appeared on the dental market, study of clinical efficacy of which is of great interest [2,22,25].

The study objective was to evaluate the clinical efficiency of use of modern remineralizing formulas: "Enamel-sealing liquid" (original name "Tiefenfluorid", Humanchemie, Germany), "FluoroLux" (TechnoDent, Russia), "InnoDent" (PLC, "InnoDent", Kazakhstan) and Clinpro™ XT Varnish (3M ESPE, Germany) in treatment of focal demineralization.

Material and methods. Clinical studies were carried out based on the dental clinic of FSBEI HE Kuban State Medical University of the Ministry of Health of Russia. In total, 36 volunteers aged 18-30 years old with a satisfactory level of oral hygiene without severe concomitant somatic pathology took part in study. Volunteers were informed about the upcoming preventive measures and gave their consent to them.

In the framework of a clinical study, four groups of patients were formed (n=36): Group 1 (n=9 people, 30 teeth with enamel caries diagnosis); Group 2 (n=9 people, 29 teeth with enamel caries diagnosis); Group 3 (n=9 people, 28 teeth with enamel caries diagnosis); Group 4 (n=9 people, 31 teeth with enamel caries diagnosis).

All patients underwent professional oral hygiene, oral hygiene training using standard techniques and home care recommendations.

Patients of the first group underwent deep fluoridation with the drug "Enamel-sealing liquid" (Humanchemie, Germany) according to the method of Professor A. Knappvost. Liquid No. 1 was rubbed onto clean surfaces of the teeth with microbrush for 30 seconds, then liquid No. 2 was applied for 30-60 seconds. The result is the decomposition of complex salts, deep penetration of active ions into the enamel, which form submicroscopic crystals of calcium fluoride, magnesium fluoride and copper hydroxyfluoride, enclosed in a high molecular weight polymer of silicic acid, which protects the crystals from leaching. Formed dense polymer substance has alkaline reaction, protects the enamel, has a long-term bactericidal activity [14,17,18]. Patients underwent two deep fluoridation procedures with a multiplicity of two weeks.

Patients of the second group underwent deep fluoridation with the drug "FluoroLux" (TechnoDent, Russia) according to the method of Professor A. Knappvost. Deep fluoridation with "FluoroLux" is based on chemical reactions that occur during the sequential processing of hard tooth tissues with a solution of magnesium and copper fluoride silicates and a suspension of highly dispersed calcium hydroxide, which leads to the formation of a fluorosilicate complex. The complex spontaneously disintegrates with the formation of microcrystals of calcium fluoride, magnesium, copper and polymerized silicic acid. The resulting CaF2 nanocrystals have a size of 50 A, therefore they penetrate well into the enamel pores with a diameter of 100 A. Fluoride crystals are located on the surface and deep inside the enamel in a thixotropic silica gel, which protects them from leaching [20]. Patients underwent two deep fluoridation procedures with a multiplicity of two weeks.

Patients of the third group underwent a biomineralization procedure with the drug "InnoDent" (PLC, InnoDent, Kazakhstan). The method is based on the biomimetic restoration of enamel using an analogue of the natural protein amelogenin (matrix), which regulates the process of building hydroxyapatite crystals in the enamel, followed by remineralization with essential trace elements from the patient's natural environment (saliva) [11,16]. Procedure: after carrying out professional hygiene, teeth are isolated from saliva, treated with 2% chlorhexidine solution, to open micropores, a 37% solution of orthophosphoric acid is applied for 20 seconds, the acid is washed off with water, the surface of the teeth is dried with an air stream. InnoDent is diluted with 0.05 ml of distilled water, 1-2 drops are applied with a micro applicator, is not washed off within 5 minutes. The patients underwent a biomineralization procedure once.

Patients of the fourth group underwent remineralization using Clinpro™ XT Varnish (3M ESPE, Germany) - light-cured, hybrid, glass-ionomer material of prolonged action, which releases fluorides, calcium, and phosphates in large quantities in the first 24 hours after application. In addition, the material on the surface of the tooth continues to be "recharged" every time the patient brushes their teeth with a paste containing fluorine, calcium, and phosphates [3,15,26]. Methodology: the cleaned surface is rinsed with water, then the remaining water is removed, the required amount of the drug is squeezed out of the dosing system (Clieker™) on the notepad for kneading, the material is kneaded for 15 seconds (working time is 2.5 minutes). Then a thin layer is applied on the surface of the tooth. The drug is polymerized by a lamp for 20 seconds, and then the surface is wiped with a wet applicator. The patients underwent a remineralization procedure once.

To assess the effectiveness of the treatment of foci of enamel demineralization with test drugs, volunteers were diagnosed using methods:

1) Laser fluorescent apparatus "DiagnoDent Pen" (KaVo);

2) Vital staining with a 2% aqueous solution of methylene blue with subsequent evaluation on the control gradation 10-point grayscale scale [21].

Comparison of the estimated indicators in the study groups was carried out using the Wilcoxon and Mann-Whitney tests. To assess the results obtained, nonparametric statistics software packages were used (StatSoft Statistica 12.0).

Results and discussion. Clinical studies of the remineralizing activity of the drugs were carried out on the basis of the laser fluorescence data obtained and vital staining indicators.

The results obtained in the study groups are given in the tables (Table 1,2). Clinical cases are presented in figures (Fig. 1-4).

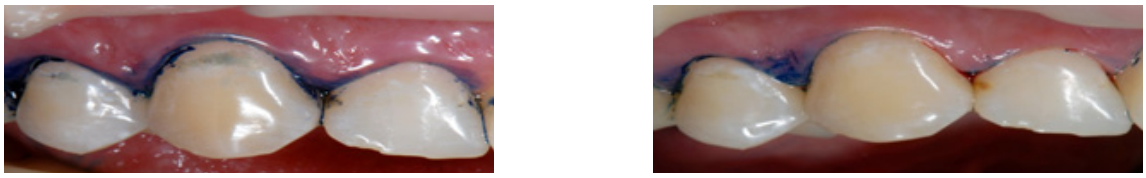

Fig. 1 The results of the vital staining of focal demineralization in the group before and after the deep fluoridation "Enamel-sealing liquid"

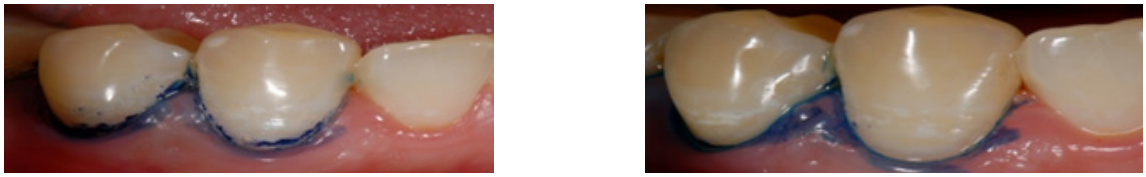

Fig. 2 The results of the vital staining of focal demineralization in the group before and after the deep fluoridation "FluoroLux"

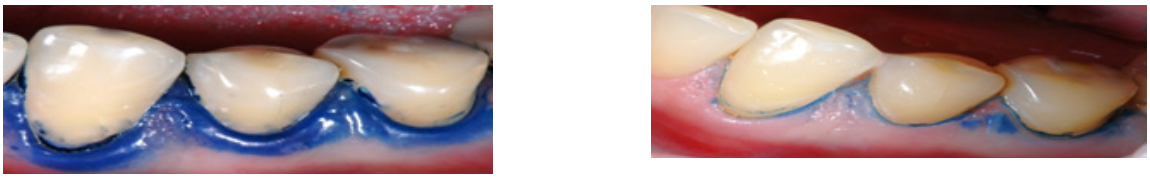

Fig. 3 The results of the vital staining of focal demineralization in the group before and after the biomineralization "InnoDent".

Fig. 4 The results of the vital staining of focal demineralization in the group before and after the remineralization "Clinpro™XT Varnish"

Table 1. Results of parameters of laser fluorescence in study groups

Groups	Periods of observation	Median (25%;75%)	Significance of difference on observation stages in the groups in 3 months	Significance of difference on observation stages between the groups in 3 months
Liquid	before	21 (18;23)	p*=0.000002	p2=0.56 p3=0.365 p4=0.449
	3 months	10 (9;11)		p2=0.134 p3*=0 p4*=0
Deep fluoroe	before	21 (18;24)	p*=0.000002	p1=0.56 p3=0.15 p4=0.21
	3 months	11 (10;11)		p1=0.134 p3*=0 p4*=0
Biomineralization	before	21 (19;24)	p*=0.000002	p1=0.365 p2=0.15 p3=0.935
	3 months	14 (14;15)		p1*=0 p2*=0 p3=0
Clin Pro	before	21 (18;24)	p*=0.000002	p1=0.449 p2=0.21 p3=0.935
	3 months	13 (12;15)		p1*=0 p2*=0 p3*=0

p - statistically significant difference is taken at p<0.05*

Based on the data presented in Table 1, it was found that all patients of the three groups, before treatment, showed staining of enamel demineralization foci of blue color of varying intensity in 100% of cases, with the minimum value on a 10-point scale being 2-3 points, maximum - 8. Indicators of the laser-fluorescent method with the "DiagnoDent Pen" apparatus before treatment ranged from 18 to 24 units.

Comparison of the performance of each group after 3 months of observation was carried out using the Wilcoxon test. Comparison of the performance of the groups between themselves before and after treatment was performed using the Mann-Whitney test. The confidence level was accepted at $p \leq 0.05$.

When comparing the laser fluorescence values in the pretreatment groups, no statistically significant difference was found. This indicates the closeness of the values of medians in all groups before treatment ($p>0.05$).

The enamel laser fluorescence index, expressed in units, with high statistical significance decreased in all groups after 3 months compared with the data before treatment (p=0.000002). In the first group, the median decreased by 52%. In the second group, the median decreased by 47%. In the third group, the median decreased by 33%. In the fourth group, the median decreased by 38%.

When comparing the first and second groups after 3 months, no statistically significant differences were found. When comparing the data of the first and second groups with the data of the third and fourth groups, a statistically significant difference was revealed. That is, in the groups of patients in whom deep fluoridation was performed with "Enamel Sealing Liquid" and "FluoroLux", the rate of laser fluorescence was significantly lower in 3 months than in the groups in which the biomineralization procedure was performed with "InnoDent" and remineralization with "Clinpro™ XT Varnish". When comparing the data of the third and fourth groups after 3 months, a statistically significant difference was revealed. That is in the groups of patients who underwent remineralization with "Clinpro™ XT Varnish" the indicator of laser fluorescence was significantly lower compared with the data after biomineralization with "InnoDent" (Table 1).

When comparing the vital staining values in the pretreatment groups, no statistically significant difference was found. This indicates the closeness of the values of medians in all groups before treatment ($p>0.05$).

The vital staining index, expressed in points, with high statistical significance decreased in all groups after 3 months compared with the data before treatment (p=0.000002).

When comparing the first and second groups after 3 months, no statistically significant differences were found. When comparing the data of the first and second groups with the data of the third and fourth groups, a statistically significant difference was revealed. That is, in the groups of patients in whom deep fluoridation was performed with "Enamel Sealing Liquid" and "FluoroLux", the rate of vital staining was significantly lower than in the groups in which the biomineralization procedure was performed with "InnoDent" and remineralization with "Clinpro™ XT Varnish". When comparing the data of the third and fourth groups, a statistically significant difference was revealed. That is in the groups of patients who underwent remineralization with "Clinpro™ XT Varnish" the indicator of vital staining was significantly lower compared with the data after biomineralization with "InnoDent" (Table 2).

Table 2. Results of parameters of vital staining in study groups

Groups	Periods of observation	Median (25%;75%)	Significance of difference on observation stages in the groups in 3 months	Significance of difference on observation stages between the groups in 3 months
Liquid	before	6 (6;8)	p*=0.000002	p2=0.686 p3=0.842 p4=0.485
	3 months	3(2;4)		p2=0.169 p3*=0 p4*=0.000002
Deep fluoroe	before	6 (6;8)	p*=0.000002	p1=0.686 p3=0.84 p4=0.76
	3 months	3 (2;3)		p1=0.169 p3*=0 p4*=0.000047
Biomineralization	before	6 (6;8)	p*=0.000002	p1=0.842 p2=0.84 p4=0.615
	3 months	4 (4;5)		p1*=0 p2*=0 p4=0.000001
Clin Pro	before	6.5 (6;8)	p*=0.000002	p1=0.485 p2=0.76 p3=0.615
	3 months	3 (3;4)		p1*=0.000002 p2*=0. 000047 p3*=0.000001

p - statistically significant difference is taken at p<0,05*

According to the results of clinical data, the high efficiency of the remineralizing activity of deep fluoridation was established, both when using "Enamel-Sealing Liquid" and when using "Fluor-Lux". First, the liquid could penetrate deeply into the structure of enamel and to perform prolonged release of fluoride, calcium, and copper ions. Secondly, it has a long-lasting antimicrobial and remineralizing effect, which is indirectly confirmed by the obtained clinical results. Conducting deep fluoridation in the treatment of enamel caries in the "white spot" stage stops the process of demineralization, prevents further progression of the pathological process, and contributes to increasing the resistance of hard dental tissues. The presented dosage forms for deep fluoridation in the treatment of enamel caries reliably show remineralizing activity comparable with each other, exceeding the control indicators, and can be interchanged when used at the discretion of the attending physician.

According to the results of laser fluorescence and vital staining after 3 months, it was found that the use of "ClinproTM XT Varnish" gives a positive effect in reducing focal enamel demineralization. This may be because the drug acts mainly as a physical barrier that prevents direct contact of acidic substances with the enamel surface. It should be noted that in the group where "ClinproTM XT Varnish" was used, patients 'strict adherence to recommendations on the use of fluoride-containing oral hygiene products was required, which should "recharge" the film formed on the enamel surface and accumulate fluorine ions in her.

According to the data of laser fluorescence and vital staining after 3 months, it was found that the biomineralization procedure with the "InnoDent" drug gives a moderate positive effect in reducing the focal demineralization of enamel. This may be because the enamel growth process (enamelogenesis) is one of the slowest morphogenetic processes, which requires more time to complete than during the period of embryonic enamel formation. The results obtained in the group in which the method of biomineralization with the "InnoDent" drug was carried out in our opinion was influenced by the following factors: as the enamel ripens, the enamel protein profile changes. At the stage of formation, the ratio of amelogenins and enamelins is 9:1, and in mature enamel it reaches 1:1. In this regard, the use of this drug may be more effective in patients during the period of ripening enamel.

Conclusion. Based on the obtained results, it can be concluded that modern remineralizing drugs with advanced technology can be effectively used as therapeutic and prophylactic agents for enamel caries (focal demineralization).

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SUMMARY

COMPARATIVE EVALUATION OF THE CLINICAL EFFICACY OF MODERN REMINERALIZING DRUGS IN THE TREATMENT OF ENAMEL CARIES (FOCAL DEMINERALIZATION)

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The emergence of new concepts and mechanisms for remineralization is of great interest to study. The study objective was to evaluate the clinical efficiency of use of modern remineralizing formulas: “Enamel-sealing liquid” (“Tiefenfluorid”, Humanchemie, Germany), “FluoroLux” (TechnoDent, Russia), “InnoDent” (PLC, “InnoDent”, Kazakhstan) and Clinpro™ XT Varnish (3M ESPE, Germany) in treatment of focal demineralization. According to the number of studied drugs in the framework of a clinical study, four groups of patients with a diagnosis of caries of enamel were formed (n=36): Group 1 (n=9 people, 30 teeth); Group 2 (n=9 people, 29 teeth); Group 3 (n=9 people, 28 teeth); Group 4 (n=9 people, 31 teeth). Evaluation of the clinical efficacy of enamel caries treatment was carried out using 1) vital staining with a 2% aqueous solution of methylene blue followed by evaluation using a control gradation 10-point grayscale; 2) laser-fluorescence method using the apparatus “DiagnoDent Pen” (KaVo). Statistical processing of the obtained results was performed using the Wilcoxon and Money-Whitney test. The confidence level was considered at $p \leq 0.05$. According to the data of laser fluorescence of enamel and vital staining, the median index with high statistical significance decreased in all groups after 3 months compared with the data before treat-

ment. Thus, all the studied drugs showed remineralizing activity in the elimination of enamel demineralization foci. This study is the basis for the search and development of new remineralizing compounds for the prevention and treatment of focal enamel demineralization.

Keywords: InnoDent, demineralization, enamel-sealing liquid.

РЕЗЮМЕ

СРАВНИТЕЛЬНАЯ ОЦЕНКА КЛИНИЧЕСКОЙ ЭФФЕКТИВНОСТИ СОВРЕМЕННЫХ РЕМИНЕРАЛИЗИРУЮЩИХ ПРЕПАРАТОВ В ЛЕЧЕНИИ КАРИЕСА ЭМАЛИ (ФОКУСНАЯ ДЕМИНЕРАЛИЗАЦИЯ)

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Изучение концепции реминерализации зубочелюстной системы является актуальным научно-исследовательским направлением.

Целью исследования явилась оценка клинической эффективности применения современных реминерализующих режимов, применяемых в лечении очаговой деминерализации, в частности «Enamel-sealing liquid» («Tiefenfluorid», Humanchemie, Германия), «FluoroLux» (ТехноДент, Россия), «InnoDent» (PLC, «InnoDent» Казахстан) и ClinproTM XT Varnish (3M ESPE, Германия).

Статистическая обработка и детализированная оценка полученных данных выявила, что в группе пациентов с применением «Enamel-sealing liquid» и «FluoroLux» скорость витального окрашивания была значительно ниже, чем в группах, в которых процедура биоминерализации проводилась при помощи «InnoDent» и реминерализации при помощи «ClinproTM XT Varnish». В группах пациентов, прошедших реминерализацию с помощью «ClinproTM XT Varnish», показатель витального окрашивания был значительно ниже в сравнении с данными после биоминерализации «InnoDent».

Получены данные выявили эффективность применения «InnoDent», однако авторы рекомендуют проведение более обширных исследований в этом направлении.

რეზიუმე

თანამედროვე მარემინერალიზებელი პრეპარატების კლინიკური ეფექტურობის შედარებითი შეფასება მინანქრის კარიესის მკურნალობაში (ფოკუსური დემინერალიზაცია)

ზ.სოლოვიოვა, ე.ზაპოროჟსკაია-აბრამოვა, ა.ადამჩიკი, ა.გუშჩინი, ს.რისოვანნი, ი.მანუკიანი

კუბანის სახელმწიფო სამედიცინო უნივერსიტეტი, რუსეთის ფედერაცია

ყბა-კბილთა სისტემის რემინერალიზაციის კონცეფციის შესწავლა წარმოადგენს აქტუალურ სამეცნიერო-კვლევით მიმართულებას. კვლევის მიზანს წარმოადგენდა იმ თანამედროვე მარემინერალიზებელი რეჟიმების კლინიკური ეფექტურობის შეფასება, რომლებიც გამოიყენება კეროვანი დემინერალიზაციის მკურნალობაში, მაგალითად, “Enamel-sealing liquid” («Tiefenfluorid», Humanchemie, გერმანია), «FluoroLux» (ტექნოდენტი, რუსეთი), «InnoDent» (PLC, «InnoDent», ყაზახეთი) და ClinproTM XT Varnish (3M ESPE, გერმანია).

მიღებული შედეგების სტატისტიკური დამუშავებით და დეტალური შეფასებით გამოვლინდა, რომ პაციენტებში, რომლებიც იყენებდნენ «Enamel-sealing liquid»-ს და «FluoroLux»-ს, ვიტალური შეღებვის სიჩქარე იყო მნიშვნელოვნად ნაკლები, ვიდრე ჯგუფებში, სადაც ბიორემინერალიზაცია ტარდებოდა «InnoDent»-ით, რემინერალიზაცია კი - «ClinproTM XT Varnish»-ით. პაციენტების ჯგუფებში, რომლებშიც რემინერალიზაცია ჩატარდა «ClinproTM XT Varnish»-ით ვიტალური შეღებვის სიჩქარე იყო მნიშვნელოვნად ნაკლები, «InnoDent»-ით ბიორემინერალიზაციასთან შედარებით.

მიღებული შედეგებით გამოვლინდა «InnoDent»-ის გამოყენების ეფექტურობა, თუმცა, ავტორები რეკომენდებულად თვლიან ფართო კვლევების ჩატარებას ამ მიმართულებით.

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ELECTROPHYSIOLOGICAL CORRELATES OF MASTICATORY MUSCLES IN NASAL AND ORONASAL BREATHING MODES

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Despite various approaches that miscellaneous orthodontic trends may suggest, main goal of an orthodontist is early prevention of orthodontic abnormalities. In fact, more frequently, specialists have to deal with a result rather than a cause of orthodontic abnormalities making long-lasting effects less achievable and likelihood of relapse greater. Mechanical expansion of narrowed jaws or adjustment of malocclusion is not feasible unless accompanied by proper neuromuscular balance. Therefore, main goal faced by an orthodontist is to prevent this type of abnormalities and, if they do exist, not only deliver effective therapy, but, rather, keep the, achievement long-term without using any appliances.

Contemporary dentistry regards masticatory apparatus as an integral functional part of organs belonging to this system. As muscles of mastication, temporomandibular joint, periodontium and teeth are normally functionally interconnected, change in one part of this system affects functioning of the rest, to a certain extent.

One of the necessary precondition for physiologic occlusion is relaxation of masticatory muscles whose achievement may require application of transcutaneous electrical nerve stimulation.

One of the requirements for neuromuscular balance of the masticatory apparatus is a good tongue posture where the tongue rests against the palate thereby promoting proper growth of the maxilla, which per se is one of the main preconditions for early prevention of maxillofacial discrepancies. Good tongue posture has utmost importance for the proper development of facial skeleton and occlusion [12]. Abnormal breathing pattern due to mechanical factors, allergies or facial structure can disrupt proper tongue-palate relationship in the very childhood, drop it away from the palate and lead to an open bite [15]. As it turns out, nasal breathing and tongue posture closely correlate. Changing this relationship can cause collapse of various degrees in the entire stomatognathic system and lead to problems connected with vertical growth of upper and lower jaws, narrowing of the air passageways and adversely affecting quality of life overall [16].

As we have mentioned in the earlier publication [1], nasal breathing has a significant impact on the qualitative and quantitative physiological correlates of the masticatory muscles. Comparing nasal and mouth breathing modes demonstrates that, in nasal breathing, masseter and temporalis muscles activity is homogenous and symmetric, while, in oral breathing, their contractility is reduced and significantly dissociated.

In the context of this background, it is particularly interesting to see how electrophysiologic characteristics of masticatory muscles differ in nasal and oronasal breathing modes. Interest over this issue, as well as over the one highlighted in the first study, has been heightened lately among researchers and specialist doctors [9], although, to date, electromyography – the only objective research method for these muscles [13] – has not been exhaustively utilized in different studies which makes the stated correlation particularly attractive for research [17].

Our interest in electromyographic indicators of masseter and temporalis muscles, in turn, is twofold: how symmetrically they contract during maximal clenching on the cotton roll, i.e. when dentoalveolar proprioception is minimal, and how powerfully they contract in the same test in patients with different breathing patterns [14,19].

Aim of the study was to assess electrophysiologic characteristics of masticatory muscles, right and left temporalis and right and left masseter, in nasal and oronasal breathing modes.

Material and methods. The study was conducted on the group of 30 female volunteers who were referred to the clinic for orthodontic problems. Conflict of interests was ruled out. The research was carried out within a frame of population-based study.

All subjects enrolled in the study had permanent dentition with all second molars and a minimum of 28 natural teeth present in total. None of them had clinically manifested systemic somatic, neurological or endocrine disorders or those of nasal cavity, paranasal sinuses or tonsillar disorders. Written informed consent was obtained from all subjects. To narrow the selection of subjects eligible for the study, we applied an electromyographic protocol of normalized electromyographic recording during maximal voluntary clenching of teeth on a cotton roll [2].

Electrophysiologic study of masseter and temporalis muscles was conducted bilaterally (right and left) using an apparatus Easymyo (Italy).

Silver/silver-chloride bipolar electrodes for the study of masseter muscles were placed on the external surfaces [5] of the cheeks on a proper side parallel to the muscle fibers over the most easily palpated protuberance during maximal voluntary contraction of clenching. Reference electrode was placed on the forehead [18]. The area of the skin was cleaned with an alcohol-based solution prior to electrode placement. Electrodes were spaced at 22 mm intervals [6,10].

Electromyographic activity was recorded using four channels of eight-channel electromyograph (Easymyo). Signal was digitally filtered and averaged over 3000 ms. Electrical activity of the right and left masseter and temporalis muscles was measured in microvolts (μV) using mean value for each muscle [3].

Electromyographic potentials were standardized according to Ferrario. To block dentoalveolar proprioception and determine the degree of muscular contractility, two 10 mm-thick cotton rolls were placed between the second premolar and the first molar of the mandible and electromyogram of maximal voluntary contraction of clenching was recorded for 3 sec [4]. Subjects were asked to repeat the same act using their own occlusion [8] where occlusal contact had indirect effect on muscle tone [7]. We adopted the first test as a standard (100%) where muscle status is free from dentoalveolar proprioceptive effects [11].

Results and discussion. The table below contains indicators of electrophysiologic characteristics (μV, $P<0,001$) of masticatory muscles, right and left temporalis and right and left masseter, in nasal and oronasal breathing modes.

Table 1. Indicators of electromyographic activity of right and left temporalis and masseter muscles

Mode of breathing	Right temporalis	Left temporalis	Right masseter	Left masseter
Nasal	188 ±4,3 μV	191±4,0 μV	185 ±3,3 μV	186 ±3,9 μV
Oronasal	150±3,3 μV	140±2,3 μV	87±2,9 μV	90 ±3,0 μV

As Table 1 shows, in subjects with nasal breathing, electromyographic indicators fall within standardized range. In the group of subjects with oronasal breathing electrical activity of masseter and temporalis muscles is asymmetrical. Asymmetry between sides and unevenness between muscle pairs during normalized muscle activity may result from functionally unstable occlusion. When teeth clench on a cotton roll, dentoalveolar proprioceptive signal is too weak to affect electromyographic indicators offering possibility to obtain data solely on muscle activity during maximal voluntary contraction. Indicators of the degree of mean muscle contractility recorded as a result of standardized maximal voluntary contraction show that, in nasal breathing, muscle activity is homogenous and symmetric in the right and left masseter and temporalis muscles, while, in oronasal breathing, it becomes more dissociated with electrical activity more pronounced in temporalis muscles. Electrophysiologic activity and, consequently, contractility of both temporalis and masseter muscles are reduced compared to subjects with nasal breathing which must be caused by recruitment of less excitable motor units.

Results yielded by the study suggest the significance of the state of neuromuscular balance of the masticatory apparatus in the assessment of orthodontic status and possibility of applying electromyographic indicators to estimate the degree of orthodontic dysfunction and develop individualized treatment plans. Study continues in this respect.

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SUMMARY

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Results yielded by the study suggest the significance of the state of neuromuscular balance of the masticatory apparatus in the assessment of orthodontic status and possibility of applying electromyographic indicators to estimate the degree of orthodontic dysfunction and develop individualized treatment plans. Study continues in this respect.

Keywords: right and left temporalis muscles, right and left masseter, nasal breathing mode, oronasal breathing mode.

РЕЗЮМЕ

ЭЛЕКТРОФИЗИОЛОГИЧЕСКИЕ КОРРЕЛЯТЫ ЖЕВАТЕЛЬНОЙ МУСКУЛАТУРЫ ПРИ НОСОВОМ И ОРОНАЗАЛЬНОМ РЕЖИМАХ ДЫХАНИЯ

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Тбилисский государственный медицинский университет, [1]кафедра челюстно-лицевой хирургии и хирургической стоматологии для детей и подростков; [2]кафедра физиологии; [3]Стоматологическая клиника и Учебно-исследовательский центр «УниДент», Грузия

Целью исследования явилась оценка электрофизиологических параметров жевательной мускулатуры - правой и левой височных, правой и левой жевательных мышц - при носовом и ороназальном (смешанном) режимах дыхания.

Исследована группа женщин-добровольцев (n=30), которые поступили в клинику с ортодонтическими проблемами. Конфликт интересов отсутствовал. Исследование проводилось в рамках общественного исследования.

У лиц с носовым дыханием электромиографические показатели находятся в диапазоне физиологической нормы. В группе исследуемых с ороназальным (смешанном) дыханием электрическая активность жевательных и височных мышц была асимметрична. Асимметрия между сторонами и неравномерность между парами мышц при нормальной мышечной активности, по всей вероятности, является результатом функционально нестабильной окклюзии. При сжимании зубов о ватный валик зубочелюстной дентоальвеоллярный сиганал слишком слаб для влияния на электромиографические показатели, что дает возможность получить конкретные данные о мышечной активности во время максимального произвольного сокращения. Индикаторы степени сократимости мышц, зарегистрированные при стандартизованном максимальном произвольном сокращении указывают, что при носовом дыхании мышечная активность однородна и симметрична в правой и левой жевательных и височных мышцах; при ороназальном дыхании она становится более диссоциированной, с более выраженной электрической активностью височных мышц. Электрофизиологическая активность и, как следствие, сократимость как височных, так и жевательных мышц снижены в сравнении с субъектами с носовым дыханием, что, очевидно, вызвано уменьшением включенных в возбуждение высокопороговых двигательных единиц.

Результаты исследования свидетельствуют о значимости состояния нервно-мышечного баланса жевательного аппарата для оценки ортодонтического статуса и возможности применения электромиографических показателей для определения степени ортодонтической дисфункции и разработки индивидуального плана лечения. Исследования в этом направлении продолжаются.

რეზიუმე

საღეჭი მუსკულატურის ელექტროფიზიოლოგიური კორელატები ცხვირისა და შერეული სუნთქვის პირობებში

[1,2,3]ა.ბაქრაძე, [1]ზ. ვადაჭკორია, [2]ი.კვაჭაძე

თბილისის სახელმწიფო სამედიცინო უნივერსიტეტი, [1]ბავშვთა და მოზარდთა ყბა–სახის ქირურგიისა და ქირურგიული სტომატოლოგიის დეპარტამენტი; [2]ფიზიოლოგიის დეპარტამენტი; [3]სტომატოლოგიის კლინიკა და სასწავლო-კვლევითი ცენტრი „უნიდენტი“, თბილისი, საქართველო

კვლევის მიზანს წარმოადგენდა საღეჭი მუსკულატურის (მარჯვენა და მარცხენა საფეთქლის კუნთები, მარჯვენა და მარცხენა საღეჭი კუნთები) ელექტროფიზიოლოგიური მახასიათებლების შეფასება ცხვირისა და შერეული სუნთქვის პირობებში.

გამოკვლეულია 30 მოხალისე ქალი, რომელთაც ორთოდონტიული პრობლემით მიმართეს კლინიკას. კვლევაში ჩართვისას გამორიცხული იყო ინტერესთა კონფლიქტი. კვლევა ატარებდა საზოგადოებრივ ცდის

ხასიათს. კვლევის შედეგების ანალიზით გაირკვევა, რომ სუბიექტებში ცხვირით სუნთქვის დროს ელექტრომიოგრაფიული პოტენციალები სტანდარტიზებული მაჩვენებლების ფარგლებშია. შერეული სუნთქვის ჯგუფში საღეჭი და საფეთქლის კუნთების ელექტრული აქტივობა ასიმეტრიულია. ასიმეტრია (მხარე) და არასტაბილურობა (კუნთთა წყვილების) ნორმალიზებული კუნთოვანი აქტივობის დროს შესაძლოა წარმოადგენდეს ფუნქციურად არასტაბილური ოკლუზიის შედეგს. ბამბის ლილვაკებზე კბილების მოჭერისას დენტოალვეოლური პროპიორეცეპციის მოქმედება მიღებულ ელექტრომიოგრაფიულ მონაცემზე მინიმალურია, რაც კონკრეტულად კუნთის აქტივობის შეფასების შესაძლებლობას იძლევა მაქსიმალური დაჭერისას.

სტანდარტიზებული მაქსიმალური კონტრაქციის პირობებში დარეგისტრირებული კუნთების საშუალო კუმშვადობის ხარისხის მაჩვენებლის მიხედვით, ცხვირით სუნთქვის პირობებში როგორც მარჯვენა და მარცხენა საკუთრივ საღეჭი კუნთების, ასევე, საფეთქლის კუნთების აქტივობა ჰომოგენური და სიმეტრიულია. შერეული სუნთქვის შემთხვევაში კი მათ შორის დისოციაცია იზრდება: უფრო გამოხატულია საფეთქლის კუნთების ელექტრული აქტივობა, საღეჭთან შედარებით; როგორც საფეთქლის, ასევე საღეჭი კუნთების ელექტროფიზიოლოგიური აქტივობა, შესაბამისად - კუმშვადობა, ცხვირით სუნთქვის ჯგუფთან შედარებით, დაქვეითებულია. აღნიშნულის მიზეზს უნდა წარმოადგენდეს აგზნებაში ჩართული მაღალზღურბლოვანი მამოძრავებელი ერთეულების რაოდენობის შემცირება.

მიღებული შედეგები მიუთითებს საღეჭი აპარატის ნერვ-კუნთოვანი ბალანსის მდგომარეობის მნიშვნელობაზე ორთოდონტიული სტატუსის განსაზღვრისათვის, ასევე, ელექტრომიოგრაფიული მახასიათებლების გამოყენების შესაძლებლობის შესახებ, მათ შორის, ორთოდონტიული დისფუნქციის ხარისხის შეფასების და მკურნალობის ინდივიდური სქემის დაგეგმვის დროს. კვლევა ამ მიმართულებით გრძელდება.

INDICATORS OF LOCAL IMMUNITY IN THE COMORBID COURSE OF CARIES AND GASTROESOPHAGEAL REFLUX DISEASE

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The prevalence of hard tooth tissue diseases (dental caries) is very high (up to 96,0%) among young people in former Soviet Union, especially in Ukraine. Diseases of the digestive system have same prevalence.

It should be noted that advertising for medical help for these diseases does not always occur on time. This is due to the reluctance of treatment to the doctor (dentist), and practice of avoiding treatment generally that begins in adolescence. Survey shows that about 63,0% of young men and 87,0% of young women take drugs for the treatment of gastrointestinal disorders and pain symptoms in dental diseases without the recommendations of a doctor (dentists) focusing solely on commercial advertising [1,2,8].

Today is clearly proven the relationship between diseases of the digestive tract and pathological processes that occur in the oral cavity [8,19,21]. It was found that oral diseases have a direct influence on the condition of the digestive system. It was proved that receptors which are located in the oral mucosa, affecting the motility and secretory activity of the digestive system. Along with this, it was found that “pathological” reflexes of internal organs have a negative influence on the oral cavity [3-5,9,15]. Caries and its complications take the leading role in the development of gastrointestinal diseases in young people. The organism of young people is influenced by constant microbial invasion and sensitization when diseases of the digestive system are combined with carious teeth [10].

However, quite often various changes can be determined in the tissues and organs of the oral cavity on the early stages of systemic diseases. This is due to the fact that in the etiology and pathogenesis of dental caries, periodontal and oral mucosa diseases play main local and systemic immunity reactions.

In the organism, there is a close relationship between the main systems, including the endocrine, nervous, hematopoietic, as well as digestive. In different investigations were found likely to increase prevalence and intensity of hard tooth tissue diseases, oral mucosa diseases in adolescents and young people with various diseases of the digestive tract [12-14,20].

A number of researchers found a higher prevalence of caries, oral mucosa diseases, periodontal diseases in children and adults with inflammatory and destructive diseases of the stomach and intestines.

Different researches shown that caries of permanent teeth in children with gastrointestinal pathology was revealed in 78.4% versus 70.6% in children without digestive diseases. Children with gastroduodenitis have higher prevalence and intensity of dental caries, hyposalivation and decrease calcium level in saliva, and acidosis of saliva. It was shown the influence of the stomach and duodenum contents with reflux on the homeostasis of the oral cavity, basic salivation, and also the activation of the demineralizing properties of saliva [22, 23].

Investigating of digestive tract diseases influence on the status of the oral cavity in adult patients, researchers pay attention to the intensity of caries, the status of periodontal tissues, the hygienic state of the oral cavity, indicators of local immunity, and some indicators of saliva. Many authors note the high intensity of caries in presence of gastritis and peptic ulcer (PU) up to 22. Such high level of dental lesion in patients with PU is explained by the gastroesophageal reflux in patients of this group.

It contributes to the throwing of gastric juice into the oral cavity, which promotes the development of caries and non-carious diseases, in particular dental erosion [18].

Gastroesophageal reflux disease is one of the most common diseases of the digestive tract [18]. Some authors believe that principal causes of developing pathologies of periodontal tissues lead to gastroesophageal reflux [4,10]. According to published data, a large number of cases of erosive and ulcerative lesions of the stomach and duodenum are accompanied by reflux disease. According to several authors [1, 7], the fact of deterioration of the hygienic state of the oral cavity in people with pathology of the digestive system can change the biochemical composition of the saliva and, in particular, affect tooth decay with caries. However, the role of saliva is currently undeniable in supporting the physiological and development of pathological processes of hard and soft tissues of the oral cavity.

It was found that patients with peptic ulcer (PU) in 98% have combined immune system deficiency. It is not known what is the primary factor of internal organs diseases with subsequent damage to the oral cavity or vice versa. Authors associate indications of immunological deviations with the localization of the ulcer, the nature and period of the course of the PU, as well as with the clinical signs of periodontitis. An important role among the components of the immune defense of the oral cavity belongs to non-specific humoral factors [6,17] produced by various cells. These include the so-called factors of natural resistance: lysozyme, lactoferrin, lactoperoxidase, mucin, interferon, some components of complement, etc. All these factors are present in saliva in significant quantities and directly involved in the destruction or suppression of the vital activity of microorganisms. These components act in a complex manner, duplicate each other, which increases the final effect of protection. However, describing the properties of saliva, researchers adhere mainly to their changes in various types of dental pathology, not taking into account the general condition of the body.

Material and methods. In total were exanimated 33 patients with dental caries in age from 18 to 25 years (average age - 20,4±0,9 years), 21 (63.7%) of them were men and 12 (36.4%) were women. The main group consisted of 17 patients who had a combined dental pathology (caries) and gastroesophageal reflux disease. The comparison group consisted of 16 people who had dental caries without the other systemic diseases. The groups were randomized by age and sex.

All patients who were included in the study evaluated the dental status, immunological analysis of blood and saliva, gastroesophageal reflux disease assessment by questionnaire.

All patients were investigated for dental status with registration of the prevalence and intensity of dental caries (DMF + dm, DMF).

All patients were investigated for dental status with registration of the prevalence and intensity of dental caries. Were determined the intensity and prevalence of dental caries (DMF + dm, DMF). The structural resistance of dental hard tissues was determined by using ERT test [16]. Hygienic status of the oral cavity was determined by using the Green-Vermillion (1964) [24] and Silness-Loe (1964) Index [28]. Papillary-marginal alveolar index (PMA) was used to determine the intensity of periodontal inflammation [27]. The intensity of gingival bleeding was evaluated by index SBI (H.P. Muhlemann, S. Son (1971) [26].

Also were conducted a questionnaire survey of 33 patients, body mass index (BMI = 22.8±2.07 kg/m²), who complained of heartburn and had endoscopic signs of gastroesophageal reflux disease (non-erosive form). Questionnaire survey was carried out by using a special questionnaire to identify the clinical symptoms of gastroesophageal reflux disease, risk factors for heartburn and the course of the disease, the nature and prevalence of esophageal manifestations.

Immunological screening was carried out on 1^{st}-2^{nd} day from the first date of application and after 6 months and includes conducting tests of I and II levels according to the requirements of World Health Organization (WHO) Memorandum. Quantify major populations and subpopulations of lymphocytes to determine their functional activity of serum immunoglobulins, the concentration of circulating immune complexes of different molecular size, the phagocytic activity of neutrophils and cytokine status.

Defining phenotype lymphoid cells conducted indirect immunofluorescent method using monoclonal antibodies produced by "Sorbent service" (Moscow, Russia) against CD 3 lymphocyte antigens, CD 4, CD 8, CD 16, CD 22, with a final count on the fluorescent microscope "LUMAM I3" 200 of each cell phenotype.

Using this method were defined the following lymphocyte subsets - CD3+ lymphocytes (T-cells) CD4 + lymphocytes (T-helper) CD8 + lymphocytes (T-cytotoxic lymphocytes / suppressor) CD16 + cells (NK-cells), CD22 + lymphocytes (B -cell).

The functional activity of T-lymphocytes was studied morphological method by determining the proliferative activity of lymphocytes in lymphocyte blast transformation reaction (LBTR) with mitogen phytohemagglutinin (PHA) from «Burroughs Welcome».

The phagocytic activity of neutrophils was assessed by the degree of absorption of latex particles with calculation Hamburg phagocytic index and phagocytic number Wright.

The study of the functional state of B-lymphocytes was carried out by determining the level of the main classes of serum immunoglobulins Ig G, Ig A, Ig M by simple radial immunodiffusion in a gel according to G., Mancini et al., 1965 [25].

The concentration of circulating immune complexes (CICs) in the serum was determined by precipitation in polyethylene glycol solution (PEG-6000) on microspectrophotometry «Specol-21» (Germany) at a length wave 450 nm. At the same time, based on the differentiated precipitation in a 2.5%, 3.5% and 7.0% PEG-6000 solution, CICs fractions with different molecular weights - large-molecular (> 19S), medium- (11-19S) and small-molecular (<11S) CICs, with the last two fractions of the CICs showed high pathogenic properties.

The obtained results were processed by statistical methods using personal computers [11].

Results and discussion. As seen from the data presented in Table 1, in patients with concomitant gastroesophageal reflux disease the prevalence of caries was significantly higher than in healthy individuals without pathology of the digestive system. Indicator of dental caries intensity (DMF) in the main group was also in 1.7 higher ($p<0,05$). The results of the stability values of hard tissue to the effects of cariogenic factors had a high positive correlation with the intensity of dental caries lesions ($r=0,68$). This confirms the presence of interconnections of manifestations of somatic pathology with the development of dental caries, especially against the background of a decrease in caries resistance.

Table 1. The prevalence rate of dental caries and tooth enamel resistance cariogenic factors in the examined persons (M±m)

Indicators	Patient groups		Probability of difference indicators
	Main group (n=17)	Comparison group (n=16)	
Caries prevalence (%)	72, 2	54, 4	
Dental caries intensity (DMF)	4,31±0,21	2,53±0,14	p <0,05
Enamel resistance	41,8±2,1	42,7±2,3	p <0,05

Table 2. Oral hygiene and periodontal tissue status indices in the examined individuals (M±m)

Indicators	Patient groups		Probability of difference indicators
	Main group (n=17)	Comparison group (n=16)	
Green-Vermillion index	1,95±0,07	1,32±0,06	P<0,05
Silness-Loe index	0,72±0,08	0,36±0,05	P<0,05
PMA, %	21,35±3,21	9,31±1,02	P<0,05
SBI index	1,53±0,05	0,62±0,04	P<0,05

Table 3. Cellular immunity status in patients with caries and gastroesophageal reflux disease (M±m)

Immunological indicators	Main group (n=17)	Comparison group (n=16)	Probability of difference indicators
Leukocytes, 10^9/l	6,15±0,27	5,87±0,32	P>0,05
Lymphocytes, %	30,20±1,65	32,30±1,31	P>0,05
$CD3^+$ lymphocytes, %	56,12±1,17	57,16±1,21	P>0,05
$CD4^+$ lymphocytes, %	40,98±1,49	36,40±1,63	P>0,05
$CD8^+$ lymphocytes, %	16,70±0,94	24,60±1,19	P<0,05
$CD4^+/CD8^+$	2,42±0,09	1,32±0,07	P<0,05
$CD22^+$ lymphocytes,%	42,40±1,47	34,96±1,23	P<0,05
$CD16^+$ lymphocytes,%	16,80±0,83	16,29±0,87	P>0,05

An objective dental examination during the initial examination was also established that in the main group were significantly higher hygienic index of Green-Vermillion and Silness-Loe in 1.6 and 2.1 times, accordingly. PMA index was 21.35±1.96%, that indicating the presence of mild gingivitis and bleeding index was 1.53±0.03 points (Table 2).

A study was also conducted of the main manifestations of gastroesophageal reflux disease in the examined individuals. Analyzing the survey data in 77.4% (13 people) of respondents, the appearance of heartburn caused significant physical exertion (including physical education classes) and the adoption of fatty foods. Overeating has led to the emergence of heartburn in 52.9% of the respondents, smoking - at 29.4%, alcohol - in 23.5%, coffee - in 29.4% of the respondents.

Survey results also indicate a high incidence of flatulence among respondents that confirm previous studies on the negative impact of bloating in the onset and progression of gastroesophageal reflux disease. Thus, 12 (70.59%) patients are periodically disturbed distension.

The assessment of indicators of the immune status in patients with caries and gastrointestinal pathology was made.

The relative populations of CD3+ lymphocytes were not significantly different in the groups of patients, the total number of leukocytes and lymphocytes also had no significant differences.

The relative number of CD4+ lymphocytes/helper-inductors in the main group and comparison group had no significant differences.

The relative number of subpopulations of CD8+ lymphocytes in the main group was significantly lower by 32.12% ($p<0.05$) relative to the comparison group. As a result, was revealed an increase of the immunoregulatory index, which indicates an imbalance of immunoregulatory subpopulations associated with the development of autoimmune disorders in gastroesophageal reflux disease.

The level of B-lymphocyte population in patients with gastroesophageal reflux disease with caries significantly exceeded the data of the comparison group by 21.5% ($p<0.05$).

The number of natural killer cells does not differ from the standard values of both and examined between the groups.

By analyzing the above data, it should be noted that the reason for the activation of T- and B-lymphocytes of peripheral blood is gastroesophageal reflux disease when it is combined with caries promotes activation of immune system cells due to the appearance in blood of autoantigens.

The high number of activated lymphocytes can also be due to a significant content of proinflammatory cytokines - TNF-α and IL-1β, the level of which is elevated in patients with gastroesophageal reflux disease and caries.

Based on the detected changes in the quantitative composition of the main lymphocyte subpopulations were studied functional activity of immune cells in patients with gastroesophageal reflux disease and dental caries (Table 4).

Table 4. The functional activity of T-lymphocytes and neutrophils in patients with caries and gastroesophageal reflux disease (M±m)

Immunological indicators	Main group (n=17)	Comparison group (n=16)	Probability of difference indicators
LBTR with PHA, %	78,30±2,39	79,80±2,13	P>0,05
Spontaneous LBTR, %	4,98±0,18	3,96±0,15	P<0,05
Phagocytic index,%	84,33±0,97	75,85±1,29	P<0,05
Phagocytic number	15,28±0,23	9,63±0,19	P<0,05

Table 5. Humoral immunity status in patients with gastroesophageal reflux disease, coupled with caries (M±m)

Immunological indicators	Main group (n=17)	Comparison group (n=16)	Probability of difference indicators
Ig G, g/L	12,95±0,45	13,52±0,71	P>0,05
Ig A, g/L	1,86±0,09	1,92±0,06	P>0,05
Ig M, g/L	1,21±0,07	1,26±0,05	P>0,05
CICs large (>19 S), c.u.	34,35±1,09	43,20±0,83	P<0,05
CICs medium (11-19 S), c.u.	44,26±1,16	36,12±2,53	P<0,05
CICs small (<11 S), c.u.	18,42±0,13	12,55±0,19	P<0,05

Table 6. Local immunity status in the oral fluid of patients with gastroesophageal reflux disease, coupled with caries (M±m)

Immunological indicators	Main group (n=17)	Comparison group (n=16)	Probability of difference indicators
Ig G, g/L	3,83±0,21	2,82±0,34	P<0,05
SIg A, g/L	0,16 ±0,01	0,31±0,02	P<0,05
CICs large (>19 S), c.u.	28,43±1,21	35,61±1,46	P<0,05
CICs medium (11-19 S), c.u.	55,46±2,31	43,36± 1,84	P<0,05
CICs small (<11 S), c.u.	35,72±2,06	22,16±1,74	P<0,05
TNF-α, pg/mL	74,6 ±3,7	53,9±3,1	P<0,05

As studies have shown, the proliferative activity of T-lymphocytes in patients with gastroesophageal reflux disease and dental caries was normal and had no significant difference between the examined groups.

The presence of gastroesophageal reflux disease in patients with tooth decay is the spontaneous activation of lymphocytes proliferation by 25.8%, which may be a manifestation of how autosensibilization to own tissues, and indicate the presence of circulating self antigens, which stimulate cell proliferation, and is a feature inherent in subclinical inflammation in the immune gastroesophageal reflux disease.

In both groups surveyed were found an increase in phagocytic activity of neutrophils, significantly higher rates of phagocytic and phagocytic index due to a prevalence and intensity of caries process.

Despite the presence of B-lymphocytosis in patients with gastroesophageal reflux disease and dental caries concentration of serum Ig G, Ig A, of Ig M in both groups surveyed did not have significant differences.

In the body of patients with gastroesophageal reflux disease caries was found increased content of the medium and small molecular CICs that had pathogenic properties (Table 5). Increasing concentrations of pathogenic CICs in patients with gastroesophageal reflux disease, coupled with caries, accompanied by increased activation of autologous peripheral blood lymphocytes, an increase of spontaneous proliferative activity of lymphocytes, is a consequence of the development of gastroesophageal reflux disease.

Thus, as a result of studies, patients with gastroesophageal reflux disease when it is combined with caries observed changes in the immune system, which are features of subclinical immune inflammation.

Also, a study was conducted of local immunity of the oral fluid of persons surveyed(Table 6).

As can be seen from the data presented in Table 6, individuals with caries and pathology of the digestive tract due to the constant chemical (acid or alkali) irritation of the oral mucosa is observed significantly higher levels of proinflammatory cytokines - tumor necrosis factor-α (TNF-α), increased IgG concentration as a result of antigen challenge and also significantly lower concentration of secretory IgA (SIg A).

Reduced concentration of the latter, in an apparent, and is one of the reasons for the large intensity and prevalence of caries in patients with gastroesophageal reflux disease.

Because of the aforementioned disorders revealed CICs imbalance in oral liquid of main examined group.

Conclusions. The study revealed a higher intensity of the carious process in patients with gastroesophageal reflux disease. It is connected with a permanent lesion mucous membrane of the oral cavity acid.

Furthermore, it was found violation in terms of concentration with increasing local immunity oral liquid proinflammatory factors and a reduced concentration of secretory IgA namely with concomitant pathologies of the gastroesophageal reflux disease (GERD).

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SUMMARY

INDICATORS OF LOCAL IMMUNITY IN THE COMORBID COURSE OF CARIES AND GASTROESOPHAGEAL REFLUX DISEASE

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Aim of the study -to determine the indices of local immunity in patients who had combined dental pathology (caries) and gastroesophageal reflux disease.

In total were exanimated 33 patients with dental caries in age from 18 to 25 years, including 21 men and 12 women. The main group consisted of 17 patients who had a combined dental pathology (caries) and gastroesophageal reflux disease. The comparison group consisted of 16 people who had dental caries without other systemic diseases.

All patients, who were included in the study was carried out the following researches: a study of the dental status, an immunological study for all patients with the determination of a quantitative assessment of the main populations and subpopulations of lymphocytes, determination of their functional activity, determination of level of serum immunoglobulins, determination of the concentration of circulating immune complexes of various

molecular sizes, phagocytic activity of neutrophils and cytokine status in serum and oral fluid, as well as assessment of the course of gastroesophageal reflux disease with questionnaires.

The results of the values of the resistance of hard tissues to the effects of cariogenic factors had a high positive correlation with the intensity of dental caries lesions. That confirms the presence of a relationship between manifestations of systemic diseases together with the development of dental caries, especially against the background of a decrease in caries resistance. In the presence of gastroesophageal reflux disease in patients dental caries detected spontaneous activation of lymphocyte proliferation by 25.8%, an increasing of the phagocytic activity of neutrophils, detected significantly higher indicators of the phagocytic number and phagocytic index, increased content of medium and small molecular circulating immune complexes with pathogenic properties. Detected significantly higher content of proinflammatory cytokines - tumor necrosis factor-α, an increased concentration of IgG as a result of antigenic stimulation, and also a significantly lower concentration of secretory IgA. It has been established that in patients with gastroesophageal reflux disease combined with dental caries observing changes in the immune system, which bear the features of subclinical immune inflammation.

The study revealed a higher intensity of the carious process in patients with gastroesophageal reflux disease, which is associated with permanent acid damage of the oral mucosa. Furthermore, it was found violation in terms of concentration with increasing local immunity oral liquid proinflammatory factors and a reduced concentration of secretory IgA namely with concomitant pathologies of the gastroesophageal reflux disease.

Keywords: caries, gastroesophageal reflux disease, local immunity, cytokines, secretory immunoglobulin A, circulating immune complexes, blood serum, oral fluid.

РЕЗЮМЕ

ПОКАЗАТЕЛИ МЕСТНОГО ИММУНИТЕТА ПРИ КОМОРБИДНОМ ТЕЧЕНИИ КАРИЕСА И ГАСТРОЭЗОФАГЕАЛЬНОЙ РЕФЛЮКСНОЙ БОЛЕЗНИ

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Цель исследования - определить показатели местного иммунитета у больных стоматологическими заболеваниями (кариес), сочетанными с гастроэзофагеальной рефлюксной болезнью.

Обследовано 33 больных (21 мужчина и 12 женщин) кариесом в возрасте от 18 до 25 лет. Основную группу составили 17 пациентов с кариесом в сочетании с гастроэзофагеальной рефлюксной болезнью, группу сравнения - 16 больных кариесом без других соматических заболеваний. Пациентам проведено исследование стоматологического статуса, иммунологическое исследование с определением количественной оценки основных популяций и субпопуляций лимфоцитов, определение их функциональной активности, уровня сывороточных иммуноглобулинов, концентрации циркулирующих иммунных комплексов различного молекулярного размера, фагоцитарной активности нейтрофилов и цитокинового статуса в сыворотке крови и ротовой жидкости, а также оценка течения гастроэзофагеальной рефлюксной болезни с использованием опросников.

При наличии гастроэзофагеальной рефлюксной болезни у больных кариесом выявлены активация спонтанной пролиферации лимфоцитов на 25,8%, повышение фагоцитарной активности нейтрофилов, достоверно более высокие показатели фагоцитарного числа и фагоцитарного индекса, повышенное содержание средне- и низкомолекулярных циркулирующих иммунных комплексов с патогенными свойствами, также достоверно более высокое содержание провоспалительных цитокинов - фактора некроза опухолей-α, повышенная концентрация IgG, как следствие антигенной стимуляции и достоверно низкая концентрация секреторного IgA. Установлено, что у больных гастроэзофагеальной рефлюксной болезнью при ее сочетании с кариесом наблюдаются изменения в иммунной системе, которые носят черты субклинического иммунного воспаления.

Проведенное исследование выявило достоверно более высокую интенсивность кариозного процесса у пациентов с гастроэзофагеальной рефлюксной болезнью, вызванную постоянным кислотным поражением слизистой оболочки полости рта. При сопутствующих заболеваниях органов пищеварения (гастроэзофагеальная рефлюксная болезнь) установлено нарушение местного иммунитета ротовой жидкости, проявляющееся в повышении концентрации провоспалительных факторов и понижении концентрации секреторного IgA.

რეზიუმე

ადგილობრივი იმუნიტეტის მაჩვენებლები კარიესის და გასტროეზოფაგური რეფლუქსური დაავადების კომორბიდული მიმდინარეობისას

ა.ბორისენკო, ტ.ტიმოხინა, ო.კონონოვა

ა. ბოგომოლეცის სახ. ეროვნული სამედიცინო უნივერსიტეტი, თერაპიული სტომატოლოგიის კათედრა, კიევი, უკრაინა

კვლევის მიზანს წარმოადგენდა ადგილობრივი იმუნიტეტის მაჩვენებლების განსაზღვრა პაციენტებში სტომატოლოგიური დაავადებით (კარიესი), რომელიც შერწყმული იყო გასტროეზოფაგურ რეფლუქსურ დაავადებასთან.

გამოკვლეულია 18-25 წლის ასაკის 33 პაციენტი (21 - მამაკაცი, 12 - ქალი) კარიესით. ძირითადი ჯგუფი შეადგინა 17 პაციენტმა კარიესით და გასტროეზოფაგური რეფლუქსური დაავადებით, შედარების ჯგუფი – 16 პაციენტმა კარიესით, სხვა სომატური დაავადებების გარეშე. პაციენტებს ჩაუტარდა სტომატოლოგიური სტატუსის კვლევა, იმუნოლოგიური კვლევა ლიმფოციტების ძირითადი პოპულაციების და სუბპოპულაციების რაოდენობრივი შეფასებით, მათი ფუნქციური აქტივობის, შრატის იმუნოგლობულინების, სხვადასხვა მოლეკულური ზომის მოცირკულირე იმუნური კომპლექსების კონცენტრაციის, ნეიტროფილების ფაგოციტური აქტივობის და ციტოკინური სტატუსის განსაზღვრით სისხლის შრატსა და პირის ღრუს სითხეში; ასევე, ჩატარდა გასტროეზოფაგური რეფლუქსური დაავადების მიმდინარეობის შეფასება კითხვარების გამოყენებით.

გასტროეზოფაგური რეფლუქსური დაავადების არ-

სებობისას კარიესის მქონე პაციენტებში გამოვლინდა ლიმფოციტების სპონტანური პროლიფერაციული აქტივობის ზრდა 25,8%-ით, ნეიტროფილების ფაგოციტური აქტივობის ზრდა, ფაგოციტების რაოდენობრივი და ფაგოციტური ინდექსის უფრო მაღალი მაჩვენებლები, პათოგენური თვისებების მქონე საშუალო- და დაბალმოლეკულური იმუნური კომპლექსების მომატებული შემცველობა. გამოვლინდა პროანთებითი ციტოკინების – სიმსივნის ნეკროზის α-ფაქტორის, IgG-ის მომატებული კონცენტრაცია, ასევე, სეკრეციული IgA-ს სარწმუნოდ დაბალი კონცენტრაცია. დადგენილია, რომ პაციენტებში გასტროეზოფაგური რეფლუქსური დაავადებით და კარიესით აღინიშნება სუბკლინიკური იმუნური ანთების ნიშნების მქონე ცვლილებები იმუნურ სისტემაში.

ჩატარებული კვლევით გამოვლინდა კარიესული პროცესის სარწმუნოდ უფრო მაღალი ინტენსივობა პაციენტებში გასტროეზოფაგური რეფლუქსური დაავადებით, რაც დაკავშირებულია პირის ღრუს ლორწოვანი გარსის მუდმივი მჟავური გაღიზიანებით. დადგენილია პირის ღრუს სითხის ადგილობრივი იმუნიტეტის დარღვევა, რაც ვლინდება პროანთებითი ფაქტორების კონცენტრაციის მატებასა და სეკრეციული IgA-ს კონცენტრაციის შემცირებაში.

STUDY OF THE HYGIENIC CHARACTERISTICS OF THE ORAL CAVITY UNDER THE COMPLEX EFFECT OF PHOTODYNAMIC THERAPY AND TSKALTUBO SPRING WATER RADON HORMESIS

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Inflammatory periodontal diseases hold the second place in the frequency and prevalence of dental diseases. Periodontitis is a disease of the tissues around the tooth in which the connection between the tooth and the gum is disrupted. The pathological process gradually damages the tooth cavity, with the loss of periodontal tissues, the tooth loses its bone support and this causes loosening of the tooth [1-5,8].

Today every third person suffers from periodontitis of some degree. Risk factors for periodontitis can be: improper oral hygiene, caries, improper occlusion, improper load on the chewing apparatus, malnutrition, especially a deficiency of proteins and vitamins [1,2,9]. Saliva is of particular importance as a natural biological environment; it plays an important role in the vitality of teeth and periodontium. Saliva maintains oral homeostasis [6,10,21].

To maintain the homeostasis of mineral metabolism in the oral cavity, it is important to have a combination of hydroxyapatites, which indicates the normal functioning of saliva, in order to maintain homeostasis of the dental tissue. For this, photodynamic therapy is very important, which does not have side effects, which is based on a special chemical preparation – photosensitizer , which is locally activated by electromagnetic radiation. Thus, a photochemical reaction occurs that affects the tissue. Radiation effect on tissues can be carried out both in oxygen and in non-oxygen environment [11-13]. The mechanism of photodynamic therapy is presented as follows: a molecule of photosensitizer that absorbs a ray quantum, passes into an excited triplet state and enters into two types of photochemical reactions.

In the case of a type 1 reaction, the direct interaction with the biological substrate occurs, resulting in the formation of free radicals.

In reactions of type 2, the interaction of an active photosensitizer and dissolved molecular oxygen occurs [11-14] oxygen-dependent interaction of substances with a ray (the so-called type 2 photodynamic therapy) was discovered in 1898 by O. Raab.

Thus, with the help of the photosensitizer “Rada Dent” and the apparatus “Photodin-K”, it became possible to treat inflammatory diseases of the oral mucosa with non-invasive methods. In this case, we will use radon inhalations and rinses with radon water, because it is the alpha radiation of radon in the water of Tskaltubo that is very important in the regulation of inflammatory processes and the maintenance of oral cavity homeostasis. As it is known, in recent years, the publications [15-17] have appeared that deny the carcinogenic effect of small doses of radiation caused by radon therapy, and, conversely, it is believed that this dose is characterized by the so-called “hormesis”, thus, our area of interest is the determination of the mechanism of action of radon hormesis and its use to maintain the homeostasis of mineral metabolism in the mouth [16,17,24]. The radioactivity of the Tskaltubo mineral water ranges around 1 nq/l, about 37 nq/m^3 [18,22]. Radon therapy has a pronounced analgesic effect on inflammatory processes in the nervous tissue. It accelerates the regeneration of nerve tissue and nerve fibers [18,24,26]. Radon possesses antiseptic, antioxidant, cytoprotective, anti-inflammatory, anti-cellulite properties. It maintains the elasticity of blood vessels, prevents the development of atherosclerosis, and reduces the risk of cardiovascular diseases. It has regenerative properties, therefore, the uniqueness of Tskaltubo water and the determination of the mechanism of action of radon are very relevant and require significant study [20,23,27].

Without treatment, periodontitis eventually leads to the destruction of the alveolar ridge, loosening of the teeth and their loss.

In connection with all of the above, our main goal is to study the method of photodynamic therapy, which has become widespread in recent decades. This method of treatment has gained popularity in the treatment of both oncological and non-oncological inflammatory diseases in dentistry and other medical fields. Photosensitizer “Rada Dent”, apparatus “Photodin-K” and inhalation of Tskaltubo water with radon and the complex

action of rinses every 6 months at the beginning, and then after 1 or 2 years for the treatment of inflammatory diseases of the oral mucosa by non-invasive methods. Both treatments allow treating mild periodontitis without the use of antibiotics and steroids. The main reason for patients seeking treatment is bleeding gums, unpleasant odor, and poor hygiene.

Material and method. Clinical and laboratory work is based on examining patients, drawing up a questionnaire with the following questions: Types of food: mainly carbohydrates, proteins, sweets, mainly fast food, frequent consumption of spicy foods, obligatory allergens, great attention was paid to oral care and hygiene, how many times one cleaned teeth and what toothpaste was used, how often it was changed and in what period of time, if there any other additional attributes for cleaning teeth were used (floss, dental floss, various rinses, etc.). At the first visit with a periodontal map was filled in, then deep scaling. Production of dental cast for teeth trays (the application of the "Rada Dent" photosensitizer was carried out using individual teeth trays). Then, photodynamic therapy begins for 3 or 5 visits, depending on the condition, visits were scheduled every other day. The application was made with a tray for 40 minutes, then the photosensitizer was washed off with a stream of water and activated with a diode laser.

The questionnaire also showed whether the patient is susceptible to: alcoholism, drug addiction, service: exposure to harmful production factors, the action of carcinogens, harmful environmental factors in the environment, as well as diseases of the gastrointestinal tract, endocrine diseases, cardiovascular diseases, cardiovascular diseases ... And, having analyzed all this and based on the data of the periodontal map, the stage and degree of development of periodontitis in the patient were determined. The age of the examined patients is approximately 18-35, 35-55 years old.

Determination of protein and non-protein endogenous SH-groups. SH - Protein and non-protein groups were determined by the Sedlack method [28].

Statistical analysis. When evaluating quantitative indicators, the mean, standard deviation was taken into account. Comparisons between groups were carried out according to Student's criteria for independent selection, and before and after the treatment - using the Student's pair test and ANOVA, for qualitative indicators - comparisons were made between groups using Fisher's exact test, and before and after treatment - using the Wilkeson test. Mathematical support is implemented using the IBM SPSS v22.0 software package.

The depth of the periodontal pocket was determined, the hygienic condition of the oral cavity was assessed by the hygienic index (Pi) - by the Fedorov-Volodkina method, the prevalence of inflammatory changes in the periodontal tissues was assessed using the papillary-marginal-alveolar index (PMA).

According to the method of Fedorov-Volodkina (Федоров А.А., Володкина В.В.) the hygienic condition of the oral cavity is defined as follows: the vestibular surface of the teeth 43, 42, 41, 31, 32, 33 is stained with the Schiller-Pisarev, Lugol or other solutions. The colored surface of the tooth crown is evaluated by a 5-point system: 1 - no coloration; 2 - coloration of 1/4 of the tooth crown; 3 - coloration of 1/2 of the tooth crown; 4 - coloration of 3/4 of the tooth crown; 5 - coloration of more than 3/4 of the tooth crown. The formula for calculating the hygiene index: mean Nh.=Σ/6, where Σ - is the sum of the points of all 6 teeth; 6 - Number of teeth to be examined according to the results of Nh, the level of oral hygiene is determined. 1,1-1,5 points - good level of hygiene; 1.6-2.0 points - satisfactory; 2,1-2,5 points - unsatisfactory; 2,6-3,4 points - bad; 3.5-5.0 points - very bad. Fedorov-Volodkina hygiene index cannot be more than 5 and less than 1 point.

To determine the PMA index, a Schiller-Pissarev staining solution was applied to the vestibular surface of the gums and its position was determined for each tooth - in the areas of the gingival cavity, free (marginal) gums and attached (alveolar) gums. The inflamed areas turned dark brown [29].

Codes for assessing the degree of gingivitis 0 - no inflammation; 1 - inflammation of the gingival papilla; 2 - inflammation of the gingival papilla and marginal gums; 3 - inflammation of the gingival papilla, marginal and alveolar gums. The formula for calculating the significance of the index [2].

$$PMA = \frac{\text{Total sum of codes} * 100\%}{3^{x}\text{Number of teeth}}$$

Complex periodontal index CPI (MMCI 1987)

The CPI is used to assess the extent of damage to periodontal tissue. The examination of the oral cavity was performed using a dental mirror, dental probe and periodontal probe (for measuring periodontal pockets).

Evaluation criteria: 0 - no signs of periodontal damage; 1 - plaque; 2 - bleeding; 3 - tartar in the lower part of the gums; 4 - periodontal pockets; loosening of 5 tooth - 2-3 degree. The presence of several signs characterizes the relatively severe degree of the disease.

The CPI is calculated by the following formula:

$$\text{CPI (individual)} = \frac{\text{The sum of the signs}}{\text{Number of examined teeth}}$$

$$\text{CPI (individual)} = \frac{\text{The sum of the CPI (individual)}}{\text{Number of examined teeth}}$$

We checked the clinical and hygienic parameters of the patients in the dynamics - from the referral to the patient's clinic until the end of treatment. We also monitored them within 10 days, 6 months and one year after the end of treatment.

We conducted a complex dental examination of 135 patients aged 18-35 years and 35-55 years with periodontal pathology. Patients including 69 women, 66 men, were divided according to age and sex (Table 2).

135 patients underwent clinical-biochemical examination before and after treatment of periodontitis.

Table 1. Interpretation of the index meaning

The index meaning	The stages of gum inflammation
< 30%	Mild
31-60%	Moderate
>60%	Severe

Table 2. Distribution of the examined persons by age and sex

Distribution of patients with periodontal tissue diseases by sex and age	**Sex women**	**Sex men**
	18-35, 35-55	**18-35, 35-55**
Total	69	66
Group I	28	26
Group II	25	24
Control	16	16

Table 3. Distribution of patients by sex

Patient group	**male**		**female**	
	The number of the examined persons	**P*(%)±m***	**The number of the examined persons**	**P*(%)±m***
Main	53	55.0±6,7	50	55,5±6,7
Control	16	45,0±7,6	16	44,4±7,6
Total	69	42,5±4,95	66	57,4±4.95

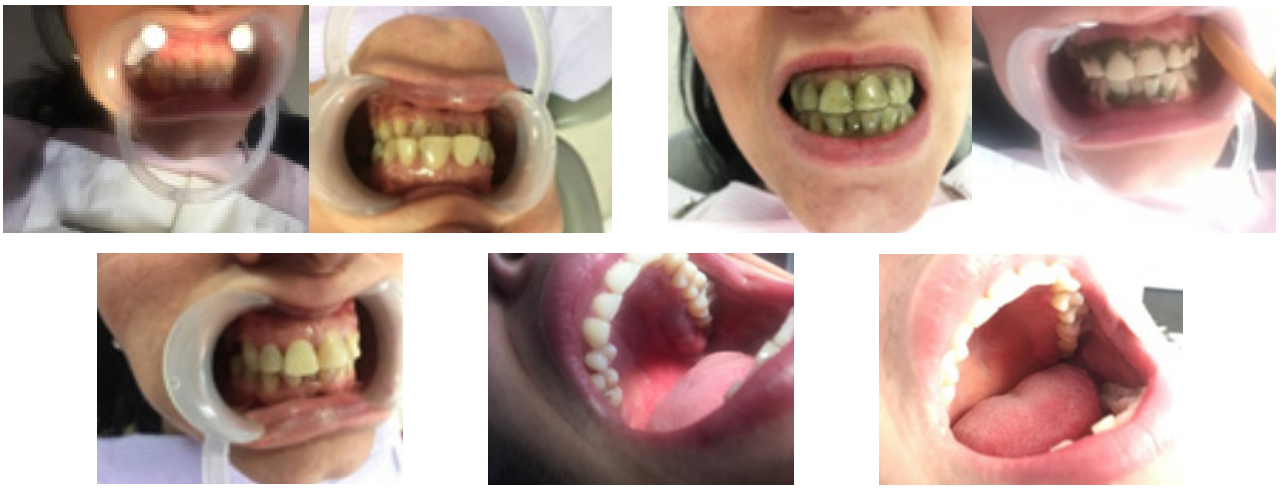

Fig. Three visits after photodynamic therapy

The main group consisted of 69 female patients who had an inflammatory process in the periodontal tissues according to which they were divided

The other 2 groups included mild periodontitis - 28, moderate - 25 and control - 16. The second group included 66 male, and 26 patients with mild periodontal disease and the second group included 22 patients with moderate inflammatory periodontitis and a control group that had healthy periodontal tissue in 16 patients.

The distribution of the examined persons into groups by gender is shown in Table 3.

We did not establish a statistically significant difference between the groups by sex ($p>0.05$), therefore, the groups with this mark can be considered comparable.

We compared the main and comparable groups with each other also by age. The average age of patients with signs of inflammation in the periodontal tissues was 25.0±0.4 years, the corresponding indicator for patients without signs of inflammation was 25.9±0.4 years. There were no statistically significant differences ($p>0.05$) compared with the mean values of age in the groups, therefore the groups can also be considered comparable in this indicator.

At the study stage, the main group of patients who had an inflammatory process in the periodontal tissues were divided into two subgroups. Patients of the first subgroup who were treated with Tskaltubo water inhalation and rinses, and patients of the second subgroup who were treated with both inhalation and rinses twice a day in the morning and evening.

As already mentioned, oral hygiene was assessed using the Hygienic Index (HI) according to the Fyodorov-Volotkina method and the Simplified Hygiene Index Green-Vermillion (1964), with patients divided by age. Hygienic condition deteriorated with age, before treatment, on visual examination, most patients showed gingival swelling, hyperemia, hypertrophy, retraction, cyanosis, bleeding Table 4.

The prevalence of inflammatory changes in periodontal tissues was assessed by the papillary-marginal-alveolar index (PMA) Parma (PARMA1960), the complex periodontal index CPI (MSM 1987) by the Russel (1956) periodontal index. Patients were divided according to age, the results of which are presented in Table 5.

Numerous experimental data are available on the fact that sulfhydryl groups of endogenous substances are involved in the prevention of primary processes of radiation damage, so we studied sulfhydryl-containing compounds in the brains of rats. As Table 7, radon inhalation appears to prevent the development of a brain disorders associated with peroxidation reactions.

Table 4. Oral Hygiene Complaints

Complaints	Main group		Control group	
	abs.	%	abs.	%
The degree of tooth loosening	3	11	1	6
Complains of uncomfortable sensations in the mouth, odor	6	18	2	11
Bleeding	11	37,0	2	11
Existence of tartar	5	16,0	-	-
Partial loss of teeth	6	17,0	1	6

Table 5. Inflammatory process of periodontal tissue by age

Periodontitis	18-22 y.	23-27 y.	18-20 y.	21-25 y.
Mild form	2.5	2.9	3.1	3.3
Moderate form	3.9	4.5	4.7	4.9
Control	1.3	1.5	1.3	1.6

Table 6. Indicator of CPI index, PMA index, Passel index Pi, changes in the indicators of the Fedorov-Volodkina index

CPI index							
Main group						Control gr.	Control gr.
Mild severity 18-30	Mild severity 30-55	Complex effect	Moderate severity 18-30	Moderate severity 30-55	Complex effect	Mild severity	Moderate severity
2.5±0,2	3.5±0,2	2.1±0,1 2.3±0,2	3.9±0,2	4.5±0,2	2.5±0,2 2.9±0,2	2.0±0,2	2.1±0,2
PMA index							
4.7±0,1	7±0,1	2.7±0,1 2.9±0,1	4.9±0,1	7.4±0,1	**2**±0,2 3.3±0,2	2.0±0,2	2.0±0,2
index Pi							
0.5±0,2	1.5±0,2	0.2±0,01 0.3±0,1			0.3±0,01 0.4±0,01	0,1±0,2	0,2±0,2
Fedorov-Volodkina index							
2.2±0,2	3.6±0,3	1.5±0,2 1.9±0,2	**2.3**±0,3	**3.8**±0,3	1.8±0,2 2±0,2	1,1±0,2	1,2±0,2

Table 7. Number of sulfhydryl groups (μm/g in tissue M±m) n=9

	KControl	After rinses and inhalation
Non-protein-compound sulfhydryl group	1,03 ±0,123	1,70 ±0,109**
Total sulfhydryl group	33,00 ±1,22	37,16 ±1,44**

*note: ** P <0.05, comparison with control*

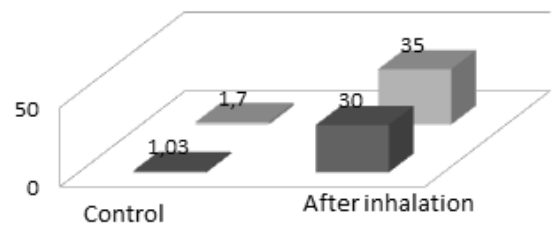

Diagram 1. Number of sulfhydryl groups

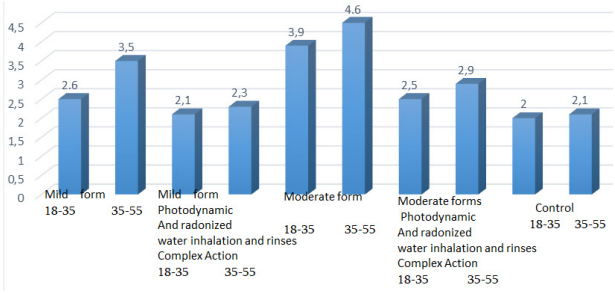

Diagram 2. The average value of the CPI index increases with age from 2.6 to 4.6

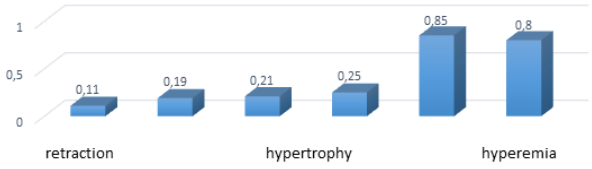

Diagram 3. The frequency of gingival retraction, hypertrophy, and hyperemia varies with age

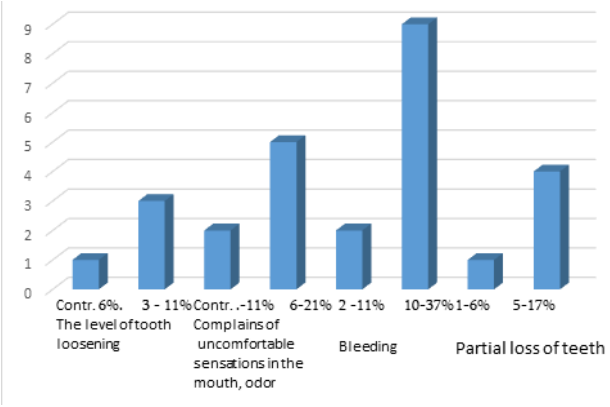

Diagram 4. The distribution of the quantitative characteristics of periodontitis is presented in the diagram of complaints

Thus, it can be assumed that radon therapy suppresses peroxidation reactions and weakens the immune system.

With the help of the «Rada Dent» photosensitizer and the «Photodin-K» apparatus, it became possible to treat inflammatory diseases of the oral mucosa with non-invasive methods. At the same time, we will use radon inhalations and rinses with radon water, and the action of «Radon Dent» and alpha radiation of radon in the water of Tskaltubo regulates inflammatory processes and maintains oral cavity homeostasis.

We can conclude that the complex action of Rada Dent and Tskaltubo water (inhalation of Tskaltubo water and its use as rinses) leads to a gradual reduction and eventual eradication of the inflammatory process in the case of periodontitis. This can be explained by the unique properties that are characteristic of «Rada Dent» and Tskaltubo water. As we have seen, the above biochemical indicators are the determinants of periodontitis in patients with periodontitis, they are markers of the degree of periodontitis and we have clearly seen the complex action. High efficiency of «Rada Dent» and radon in the water of Tskaltubo - normalization of the action of oral enzymes, slowing down the inflammatory processes in the oral cavity and finally eradication. It is these unique properties that have become triggering the treatment and inhibit the initial stage of periodontitis.

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SUMMARY

STUDY OF THE HYGIENIC CHARACTERISTICS OF THE ORAL CAVITY UNDER THE COMPLEX EFFECT OF PHOTODYNAMIC THERAPY AND TSKALTUBO SPRING WATER RADON HORMESIS

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The goal is to study the method of photodynamic therapy, which has become widespread in recent decades. This method of treatment has gained popularity in the treatment of both oncological and non-oncological inflammatory diseases in dentistry and other medical fields. We can conclude that the complex action of Rada Dent and Tskaltubo water (inhalation of Tskaltubo water and its use as rinses) leads to a gradual reduction and eventual eradication of the inflammatory process in the case of periodontitis. This can be explained by the unique properties that are characteristic of “Rada Dent” and Tskaltubo water. As we have seen, the above biochemical indicators are the determinants of periodontitis in patients with periodontitis, they are markers of the degree of periodontitis and we have clearly seen the complex action. High efficiency of “Rada Dent” and radon in the water of Tskaltubo - normalization of the action of oral enzymes, slowing down the inflammatory processes in the oral cavity and finally eradication. It is these unique properties that have become triggering the treatment and inhibit the initial stage of periodontitis.

Keywords: radon water, “Rada Dent”, mild periodontitis, medium form.

РЕЗЮМЕ

ОСОБЕННОСТИ ГИГИЕНЫ ПОЛОСТИ РТА В КОНТЕКСТЕ КОМПЛЕКСНОГО ДЕЙСТВИЯ ФОТОДИНАМИЧЕСКОЙ ТЕРАПИИ И РАДОНОВОЙ ИНГАЛЯЦИИ ВОДОЙ ЦХАЛТУБО

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Изучен метод фотодинамической терапии, который получил широкое распространение в последние десятилетия, завоевал популярность как в онкологии, так и в неонкологии, при лечении воспалительных заболеваний в стоматологии и других областях медицины. Фотосенсибилизатор «Рада Дент», аппарат «Фотодин-К» и комплексное действие ингаляции радонизированной воды Цхалтубо могут быть использованы для лечения слизистой оболочки полости рта как неинвазивный метод. Комплексное действие позволяет лечить пародонтит легкой и средней степени тяжести без применения антибиотиков и стероидов.

რეზიუმე

პირის ღრუს ჰიგიენის თავისებურებების შესწავლა ფოტოდინამიკური თერაპიის და წყალტუბოს რადონიზირებული წყლით ინჰალაციის კომპლექსური მოქმედების ფონზე

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შესწავლილია ფოტოდინამიკური თერაპიის მეთოდი, რომელიც ფართოდ გამოიყენება ბოლო ათწლეულების განმავლობაში, მოიპოვა პოპულარობა როგორც ონკოლოგიაში, ისე არაონკოლოგიაში, ანთებითი დაავადებების სამკურნალოდ სტომატოლოგიასა და მედიცინის სხვა დარგებში. ფოტოსენსიბილიზატორი “რადა დენტი”, აპარატი “ფოტოდინ-კ” და წყალტუბოს რადონიზირებული წყლის ინგალაციის კომპლექსური მოქმედება შეიძლება გამოყენებული იყოს პირის ღრუს ლორწოვანი გარსი სამკურნალოდ, როგორც არაინვაზიური მეთოდი. კომპლექსური მოქმედება იძლევა მსუბუქი და ზომიერი პაროდონტიტის მკურნალობის საშუალებას ანტიბიოტიკებისა და სტეროიდების გამოყენების გარეშე.

ОСНОВНЫЕ КРИТЕРИИ ОТБОРА ПАЦИЕНТОВ ПРИ ФОРМИРОВАНИИ ЛИСТА ОЖИДАНИЯ НА ТРАНСПЛАНТАЦИЮ СЕРДЦА

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Хроническая сердечная недостаточность (ХСН) является одной из самых распространенных патологий среди населения развитых стран. Согласно данным национальных реестров европейских стран распространенность ХСН среди взрослого населения варьирует в пределах от 2% до 5%, а число их случаев с возрастом увеличивается, сердечная недостаточность диагностируется у 10% в возрасте старше 75 лет [1,2,7-9].

В США ежегодно регистрируется более 600 000 новых случаев заболевания. Прогнозы заболеваемости показывают, что в течение последующих 20 лет количество пациентов, страдающих ХСН, возрастет вдвое [1,2,10,11].

Стандартная медикаментозная терапия, направленная на уменьшение симптомов ХСН, может обеспечить достаточное качество жизни при минимальной степени сердечной недостаточности, однако остается малоэффективной на ее терминальных стадиях. Согласно статистике Американского колледжа кардиологов и Американской ассоциации сердца, трехлетняя летальность пациентов с IV функциональным классом (ФК) по NYHA составляет почти 80% [3].

Актуальность вопросов лечения ХСН и улучшения качества жизни пациентов по сей день продолжает расти.

На фоне неэффективности медикаментозной терапии у пациентов с терминальной ХСН на первый план выходят методы хирургической коррекции, ресинхронизирующая терапия, коррекция клапанной недостаточности (митроклип, хирургическая коррекция), методы длительной механической поддержки кровообращения (ДМПК) и трансплантация сердца [4,5].

Согласно проведенному исследованию, кардиологи США в большинстве случаев назначают ингибиторы ангиотензин превращающего фермента и бета-адреноблокаторы, что не практикуется у врачей общей практики и терапевтов. Сравнительный опрос кардиологов и специалистов по лечению сердечной недостаточности выявил общие подходы к назначению терапии [6-8].

Трансплантация сердца является методом лечения пациентов в конечной стадии сердечной недостаточности (III стадия по классификации Василенко - Стражеско) с низкой толерантностью к физической нагрузке (III-IV ФК по классификации NYHA). Ограниченность ресурса донорских органов диктует необходимость тщательного отбора реципиентов и четких показаний для проведения трансплантации сердца [9-12].

По данным регистра Международного общества по трансплантации сердца и легких (ISHLT), основными показаниями для выполнения трансплантации сердца по нозологии является идиопатическая кардиомиопатия (42-49%), ишемическая кардиомиопатия (43-47%), реже - врожденные пороки, перипартальна кардиомиопатия [2-5,13,14]. Трансплантация сердца самый эффективный метод и, по сути, единственный радикальный способ лечения пациентов с терминальной стадией сердечной недостаточности [13,14].

В мире ежегодно выполняется около 5 тысяч трансплантаций, из них до 2 тысяч - в клиниках США. Однолетняя выживаемость после трансплантации сердца достигает 90%, 5-летняя - 70% (рис. 1). Эти показатели намного превышают выживаемость в сравнении с больными ХСН, получающими медикаментозное и/или любое другое хирургическое лечение [15,16].

Определение критериев, влияющих на прогноз выживаемости после хирургического лечения у пациентов с ХСН, является актуальным вопросом для принятия решения о возможности включения пациента в лист ожидания на трансплантацию сердца. Перспективность оценки непосредственных и отдаленных результатов лечения тяжелых пациентов с ХСН, с нашей точки зрения, заключается в том, что полученные результаты могут способствовать разработке новых стратегий в диагностике и лечении, способных увеличить продолжительность жизни и улучшить ее качество [7-9].

В Украине по сей день нет единых критериев по вопросам диагностики, тактики лечения пациентов с ХСН и их отборе для включения в лист ожидания на трансплантацию сердца. Рост числа пациентов с ХСН, их ранняя инвалидизация, высокая летальность и низкое качество жизни требуют внедрения в клиническую практику современных высокоэффективных методов диагностики и лечения.

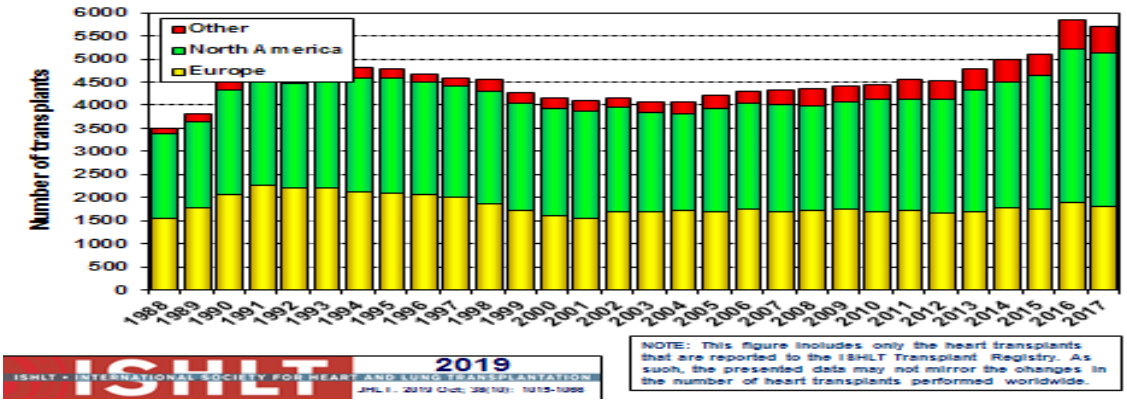

Рис. 1. Количество трансплантаций сердца по годам согласно данным международного общества трансплантации сердца и легких

Цель исследования - оптимизация критериев отбора пациентов для включения в лист ожидания на трансплантацию сердца на основании имеющихся международных данных и их внедрение в практику.

Материал и методы. Результаты исследования базируются на данных обследования и динамического наблюдения за 49 больными (медиана возраста 38 (16; 65) лет, мужчин - 44, женщин - 5), которые проходили лечение за 2008-2018 гг. в Республиканском научно-практическом центре «Кардиология» (Минск, Республика Беларусь), Центре кардиохирургии на базе КБ «Феофания» Государственного управления внутренними делами (Киев, Украина). Пациентов обследовали при первичном осмотре, спустя 3, 6 месяцев и 1 год.

I группу составили 24 пациента с хронической сердечной недостаточностью (ХСН), которые находились на поддержке кровообращения (ПК) в листе ожидания (ЛО) на ортотопическую трансплантацию сердца. Медиана возраста составляла 40,95 (18; 65) г., мужчин было 23 и одна женщина.

II группу составили 25 пациентов с ХСН, находящихся в листе ожидания на ортотопическую трансплантацию сердца без поддержки кровообращения, медиана возраста -38,56 (17; 64) года, мужчин было 21, женщин - 4. Распределение пациентов в зависимости от ФК сердечной недостаточности по NYHA представлено на рис. 2.

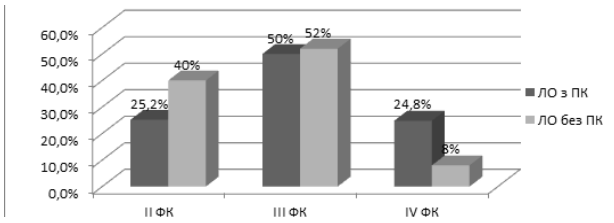

Рис. 2. Распределение пациентов в зависимости от ФК сердечной недостаточности по NYHA

Оценка ФК сердечной недостаточности по NYHA пациентов группы ЛО с ПК выявила II ФК по NYHA у 25,2% пациентов, III ФК по NYHA – у 50% пациентов, IV ФК по NYHA – у 24,8% пациентов; в группе ЛО без ПК II ФК по NYHA у 40% пациентов, III ФК по NYHA - у 52% пациентов, IV ФК по NYHA – у 8% пациентов.

Распределение пациентов в зависимости от недостаточности кровообращения представлено на рис. 3.

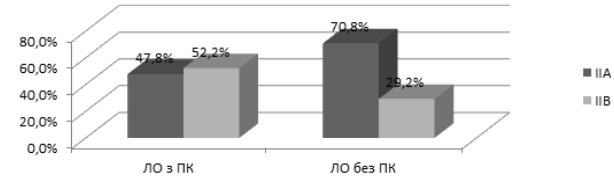

Рис. 3. Распределение пациентов в зависимости от недостаточности кровообращения

Оценивая недостаточность кровообращения (НК) у пациентов ХСН в группе ЛО с ПК НК IIA выявлена у 47,8% пациентов, НК II B - у 52,2% пациентов; в группе ЛО без ПК НК IIA - у 70,8% пациентов, НК IIB - у 29,2%. Диаграмма распределения пациентов в зависимости от нозологии заболевания в группе ЛО с ПК (n=24) представлена на рис. 4.

Рис. 4. Распределение пациентов в зависимости от нозологии заболевания в группе ЛО с ПК (n=24)

В группе пациентов ЛО с ПК (n=24): делятационная кардиомиопатия (ДКМП) - 68%, ишемическая кардиомиопатия (ИКМП) - 20%, постмиокардиосклероз - 4%, другие - 8%.

Диаграмма распределения пациентов в зависимости от нозологии заболевания в группе пациентов ЛО без ПК (n=25) представлена на рис. 5.

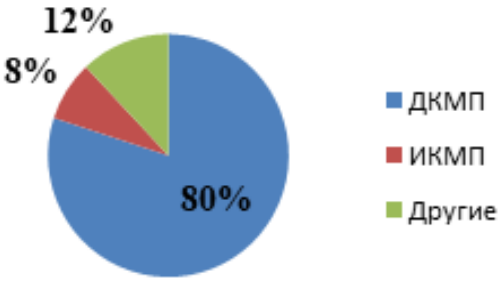

Рис. 5. Распределение пациентов в зависимости от нозологии заболевания в группе пациентов ЛО без ПК (n=25): ДКМП - 80%, ИКМП - 8%, другие - 12%

Распределение пациентов ХСН в зависимости от показателей качества жизни представлено на рис. 6.

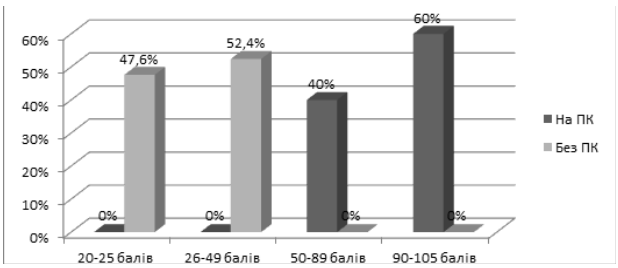

Рис. 6. Показатели качества жизни пациентов с ХСН без ПК: 20-25 баллов - 47,6% пациентов, 26-49 баллов - 52,4% пациентов; пациентов с ХСН с ПК 40-89 баллов - 40% пациентов, 90 - 105 баллов - 60% пациентов

Результаты и обсуждение. В таблице 1 приведены результаты средних значений переменных по признакам структурно-функциональных показателей левого и правого желудочков у обследованных пациентов в зависимости от вида оперативного вмешательства.

Ударный объем левого желудочка (УО ЛЖ) (М-режим) среднее значение выше во II группе пациентов ХСН в ЛО без ПК - 64 [121; 31], чем в I группе с ХСН в ЛО с ПК (n=24) - 53,9 [109; 19], p<0,0001.

УО ЛЖ (В-режим) среднее значение выше во II группе пациентов ХСН в ЛО без ПК - 63 [112; 27], чем в I группе - 55 [96; 25], p>0,05.

Конечный диастолический объем (КДО) ЛЖ (М-режим) выше во II группе пациентов с ХСН в ЛО без ПК - 281 [414; 147], чем в I группе пациентов ХСН в ЛО с ПК - 263 [371; 90], p>0,05.

Фракция выброса (ФВ) ЛЖ (В-режим) среднее значение выше в I группе пациентов ХСН в ЛО с ПК - 26 [80; 10], чем во II группе с ХСН в ЛО без ПК - 23 [33; 10], p>0,05.

Конечный систолический объем (КСО) ЛЖ (М-режим) среднее значение выше в I группе пациентов ХСН в ЛО с ПК - 235 [587; 114], чем во II группе с ХСН в ЛО без ПК 216 [309; 102], p>0,05.

ФВ ЛЖ (М-режим) среднее значение выше во II группе пациентов ХСН в ЛО без ПК - 24 [35; 12], чем в I группе с ХСН в ЛО с ПК (n=24) - 20 [29; 10], p>0,05.

ФВ правого желудочка (ПЖ): среднее значение выше во II группе пациентов ХСН в ЛО без ПК 38 [57; 21], чем в I группе 30 [50; 17], p>0,05.

TAPSE: среднее значение выше во II группе 13,3 [12; 7], чем в I группе 10,5 [13; 7], p>0,05.

КСО ЛЖ (В-режим) среднее значение выше в I группе 241 [549; 93], чем во II группе 222 (367; 93), p>0,05.

КСО ПШ: среднее значение выше в I группе 75 [269; 25], чем во II группе 48 [125; 14], p>0,05.

Переднезадний размер ПЖ: среднее значение выше в I группе пациентов ХСН в ЛО с ПК 38,5 [52; 22], чем во II группе с ХСН в ЛО без ПК 33 [50; 23], p>0,05.

КДО ЛЖ (В-режим) среднее значение выше во II группе пациентов ХСН в ЛО без ПК 289 [437; 127], чем в I группе с ХСН в ЛО с ПК 274 [380; 127), p>0,05.

КДО ПЖ: среднее значение выше в I группе 124,5 [332; 50], чем во II группе 80 [185; 25], p>0,05.

При сравнительном тестировании по признакам структурно-функциональных показателей левого и правого желудочков у обследованных пациентов отмечены корреляционные связи (рис. 7, таблица 2).

Катетеризация правых отделов сердца и исследования показателей центральной гемодинамики с определением показателей сердечного выброса (СВ), сердечного индекса (СИ), давления в полостях сердца, давления в легочной артерии (фона), центрального венозного давления (ЦВД), легочного сосудистого сопротивления (ЛСС), транспульмонального градиента давления (ТПТ). Всем потенциальным реципиентам сердечного трансплантата следует выполнять зондирование правых отделов сердца. Периодичность выполнения зондирования определяется индивидуально с учетом клинических показателей.

Таблица 1. Результаты структурно-функциональных показателей левого и правого желудочков у обследованных пациенов

Показатель	**I группа пациенты с ХСН в ЛО на ПК (n=24)**	**II группа пациенты с ХСН в ЛО без ПК (n=25)**	**F-критерий**	**p-level**	**Критерий Вандер Вардена (χ^2)**	**p-level**
УО ЛЖ (М-режим)	53,9 [109; 19]	64 [121; 31]	4,1396	0,0479	0,5859	0,4440
УО ЛЖ (В-режим)	55 [96; 25]	63 [112; 27]	3,3947	0,0722	0,0934	0,7599
КДО ЛЖ (М-режим)	263 [371; 90]	281 [414; 147]	2,7984	0,1015	1,4198	0,2334
ФВ ЛЖ (В-режим)	26 [80; 10]	23 [33; 10]	2,0763	0,1567	2,0714	0,1501
КСО ЛЖ (М-режим)	235 [587; 114]	216 [309; 102]	1,6832	0,2013	1,2969	0,2548
ФВ ЛЖ (М-режим)	20 [29; 10]	24 [35; 12]	0,9117	0,3449	1,1016	0,2939
ФВ ПЖ	30 [50; 17]	38 [57; 21]	0,8183	0,7658	1,2783	0,2582
TAPSE	10,5 [13; 7]	13,3 [12; 7]	0,6387	0,4285	1,0663	0,3018
КСО ЛЖ (В-режим)	241 [549; 93]	222 (367; 93)	0,1867	0,6678	0,0291	0,8645
КСО ПЖ	75 [269; 25]	48 [125; 14]	0,1493	0,7011	0,2789	0,5974
Передне-задний размер ПЖ	38,5 [52; 22]	33 [50; 23]	0,1076	0,7444	0,1171	0,7322
КДО ЛЖ (В-режим)	274 [380; 127)	289 [437; 127]	0,0873	0,7690	0,0023	0,9614
КДО ПЖ	124,5 [332; 50]	80 [185; 25]	0,0265	0,8713	0,1672	0,6826

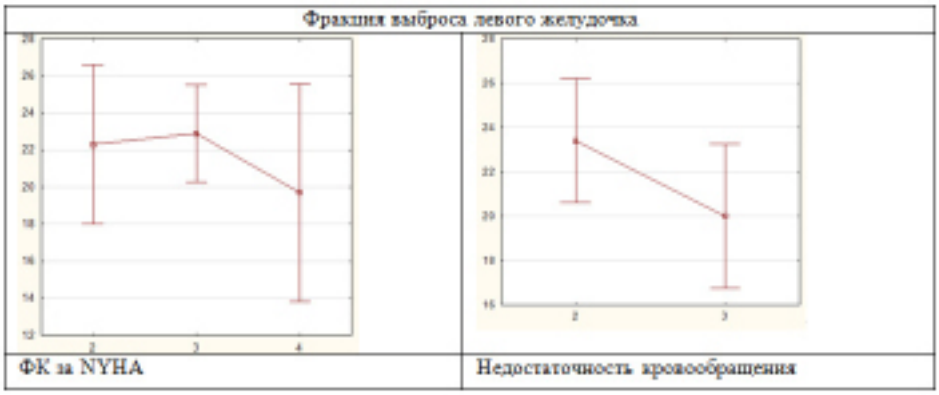

Рис. 7. Зависимость ФК по NYHA и недостаточность кровообращения от ФВ ЛЖ при значении достигнутого уровня значимости (p<0,05)

Таблица 2. Результаты средних значений переменных в исследуемых группах с учетом признаков тонометрии легочной артерии

Показатель	**I группа пациенты с ХСН в ЛО на ПК (n=24)**	**II группа пациенты с ХСН в ЛО без ПК (n=25)**	**F-критерий**	***p*-level**	**Критерий Вандер Вардена (χ^2)**	***p*-level**
Вуда	3,6 [7,2; 1]	3,2 [5; 2]	18,8145	0,0001	9,8856	0,0017
ДЛА	39,7 [57; 25]	36,6 [52; 19]	7,0570	0,0110	3,4360	0,0638
ТПГ	10,7 [17; 2]	10,3 [19; 2]	2,1592	0,1488	2,5505	0,1103

Таблица 3. Динамика изменений функциональных показателей у пациентов I и II групп

Показатель	**I группа - больные ХСН в ЛО на ПК до терапии (n=24)**	**I группа - больные ХСН в ЛО на ПК на терапии (n=24)**	**II группа - больные ХСН в ЛО без ПК до терапии (n=25)**	**II группа - больные ХСН в ЛО без ПК на терапии (n=25)**	***p*-level**
Спиро-ВЭП	12,2 [16;11]	11,8 [14;10]	13,4 [16;11,3]	14,7 [17;12]	0.0083
Тест 6 мин. ходьбой	273 [400;90]	250 [390;50]	323 [492;230]	330 [450;240]	0.7933

В таблице 2 показано, что: среднее значение ЛСС Вуда выше в I группе пациентов ХСН в ЛО с ПК - 3,6 [7,2; 1], чем во II группе с ХСН в ЛО без ПК - 3,2 [5; 2], p<0,0017. ДЛА: среднее значение выше в I группе - 39,7 [57; 25], чем во II группе - 36,6 [52; 19], p<0,0638. ТПГ: среднее значение выше в I группе пациентов ХСН в ЛО с ПК - 10,7 [17; 2], чем во II группе пациентов ХСН в ЛО без ПК - 10,3 [19; 2], p<0,1103.

У пациентов с терминальной стадией сердечной недостаточности значительно снижается максимальное потребление кислорода <14 мл/кг/мин или процент от рассчитанного максимального потребления кислорода (<50%), несмотря на медикаментозную терапию.

Максимальное потребление кислорода миокардом в группе с ПК (таблица 3). Спировелоэргометрия (Спиро-ВЭП): среднее значение выше в I группе пациентов ХСН в ЛО с ПК на терапии (n=24) -11,3 [14; 7], чем в I группе пациентов с ХСН в ЛО с ПК до терапии (n=24) - 10,7 [12,7; 9]. Наблюдается увеличение максимального потребления кислорода миокардом на 5,3%, p<0,0001.

Толерантность к физическим нагрузкам. Тест с 6-минутной ходьбой: среднее значение выше в I группе пациентов с ХСН в ЛО с ПК до терапии (n=24) - 273 [400; 90], чем в I группе пациентов с ХСН в ЛО с ПК на терапии (n=24) - 250 [390; 50]. Наблюдается снижение толерантности к физической нагрузке на 7%, p>0,05.

Результаты функциональных показателей в I группе пациентов с ХСН в ЛО с ПК к ОТС (n=24): увеличение максимального потребления кислорода миокардом на 7% и снижение толерантности к физической нагрузке на 7%. В этой группе пациентов 24 пациентам выполнена прямая трансплантация сердца.

Результаты изменений функциональных показателей у пациентов во II группе (n=25): пиковое потребление кислорода миокардом спиро-ВЭП в группе пациентов с ХСН в ЛО без ПК на лечении - 15,2 [27; 10]. Определяется увеличение максимального потребления кислорода миокардом на 5,2% (p<0,0001).

Толерантность к физической нагрузке: тест 6-минутной ходьбы во II группе при терапии - 330 [450; 240], с увеличением толерантности к физической нагрузке на 2% (p>0,05).

Таблица 4. Основные группы лекарственных препаратов, принимаемых пациентами I и II групп

Препараты, принимаемые пациентами I группы	**n (%)**	**Препараты, принимаемые пациентами II группы**	**n (%)**
ИАПФ	23(95.8%)	ИАПФ	25 (100%)
Бета-блокаторы	22 (91.7%)	Бета-блокаторы	25 (100 %)
Диуретики	24 (100%)	Диуретики	25 (100%)
Антагонисты альдостерона	24 (100%)	Антагонисты альдостерона	25 (100%)
Антиагреганты	20 (83.3%)	Антиагреганты	20 (75 %)
Сердечные гликозиды	5 (20.8%)	Сердечные гликозиды	20 (10%)
Ивабрадин	5 (20.8%)	Ивабрадин	5 (25%)
Антикоагулянты	10 (41,6%)	Антикоагулянты	5 (25 %)
Статины	11 (45.8%)	Статины	10 (40 %)
Антиаритмики (амиодарон **)**	10 (41,6%)	Антиаритмики (амиодарон)	10 (40 %)
Добутамин (допамин)	9 (37,5%)		
Симдакс (левосимендан)	8 (33,3%)		
CRTD-терапия	3 (12,5%)		
LVAD-терапия	4 (17%)		
BiVAD-терапия	1 (4,1%)		

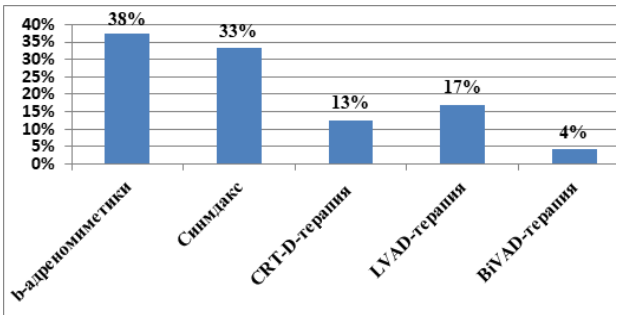

Рис. 8. Варианты поддержки кровообращения у пациентов с ХСН в ЛО на трансплантацию сердца

Результаты изменений функциональных показателей у пациентов II группы: увеличение максимального потребления кислорода миокардом на 7,2% и толерантности к физической нагрузке на 2%.

Основные группы лекарственных препаратов, принимаемых пациентами I и II групп, представлены в таблице 4.

При хорошем охвате терапии основными лекарственными средствами у пациентов I группы (n=24) дозы препаратов остаются достаточно низкими (таблица 4, рис. 11). Лишь 3 (12,5%) пациента получали ИАПФ в дозе 75-100% от целевой. 4 (17%) пациента получали ИАПФ в дозе 50-75% от максимальной. Большинство пациентов - 17 (70,5%) получали ИАПФ в дозе менее 50% от целевой. Чаще назначали препарат рамиприл - 14 пациентов, эналаприл - 10 (41,6%) пациентов. При включении в лист ожидания на трансплантацию сердца пациенты употребляли бета-блокаторы в дозе менее 50% от целевой, 3 (12%) пациента получали от 50 до 75% от целевой дозы, лишь 21 (88%) пациент принимали бета-блокаторы в дозе менее 50% от целевой. Чаще получали препарат корведилол – 20 (80%) пациентов, вторым по частоте назначения был бисопролол - 3 пациента, третьим - метопролола сукцинат - 1 пациент. Антагонисты минералокортикоидных рецепторов (АМКР) получали 24 (100%) пациента, спиронолактон – 14 (58,3%), эплеренон – 10 (41,6%) пациентов. Один (4,2%) пациент получал менее 50% от целевой дозы, 23 (95,8%) пациента получали более 50% от целевой дозы АМКР. В качестве инотропной поддержки пациентам назначали фармакологическую поддержку бета-адреномиметиками - 9 (37,5%) пациентов, добутамином - 5 (20,8%) пациентов, допамином - 4 (16,6%) пациента, синмдакс (левосимендан) - 8 (33,3%). В качестве механического моста к трансплантации сердца у 3 (12,5%) пациентов использовали CRT-D-терапию, у 4 (17%) - LVAD-терапию, в 1 (4,1%) случае - BIVAD-терапию. Данные по поддержке кровообращения представлены на рис. 8.

Анализ проведенной медикаментозной терапии пациентам во II группе с ПК (n=25) показал, что для лечения сердечной недостаточности чаще применяли диуретики, затем - ИАПФ, антагонисты минералокортикоидных рецепторов (АМКР) и бета-адреноблокаторы. Кроме того, больным ХСН назначали дезагреганты, непрямые антикоагулянты, ивабрадин, антиаритмики, статины, преимущественно, амиодарон. При хорошем охвате терапии основными лекарственными средствами у пациентов дозы препаратов остаются достаточно низкими. Только 5 (20%) пациентов получали ИАПФ в дозе 75-100% от целевой, 80% (20) пациентов - ИАПФ в дозе 50-75% от максимальной. Чаще всего назначали рамиприл – 18 (75%) пациентов, эналаприл - 7 (28%) пациентов. При включении в лист ожидания на трансплантацию сердца 4 (16%) пациента получали бета-блокаторы в дозе меньше на 50% от целевой, только 16 (64%) пациентов получали 50-75% от целевой дозы, 5 (20%) пациентов принимали целевую дозу бета-блокаторов. Карведилол получали все пациенты. Более 50% от целевой дозы АМКР получали 25 (100%) пациентов, из них 5 пациентов принимали спиронолактон, 20 – эплеренон.

Показатели тяжести состояния в исследуемых группах пациентов с ХСН в ЛО до, на медикаментозной терапии и после – ортотопической трансплантации сердца (ОТС) по функциональному классу СН по NYHA представлены на рис. 10.

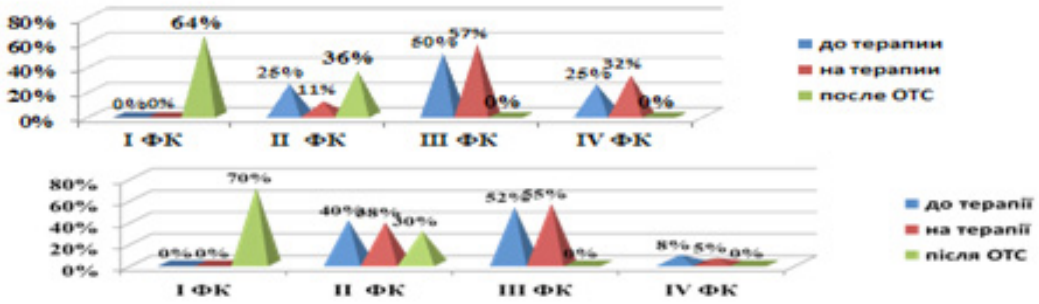

Рис. 9. Показатели тяжести состояния в I и II группах пациентов ХСН в ЛО до, во время медикаментозной терапии и после ОТС по функциональному классу СН по NYHA

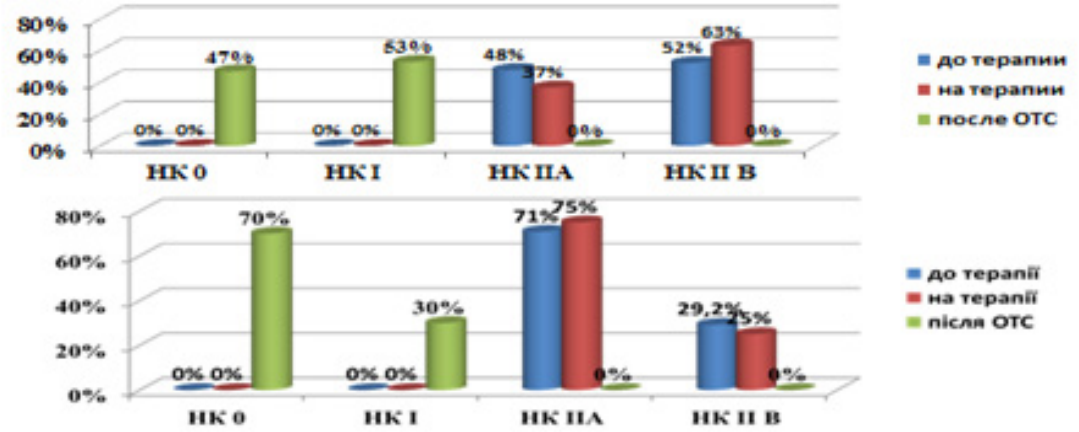

Рис. 10. Оценка тяжести состояния пациентов до, во время медикаментозной терапии и после ОТС за недостаточностью кровообращения в I группе: к терапии НК IA - в 47,8% пациентов, НК II B - в 52,2% пациентов; при медикаментозной терапии: НК IIA - в 36,8% пациентов, НК II B - в 63,2% пациентов; после ОТС: НК 0 - в 47%, НК И - в 53%

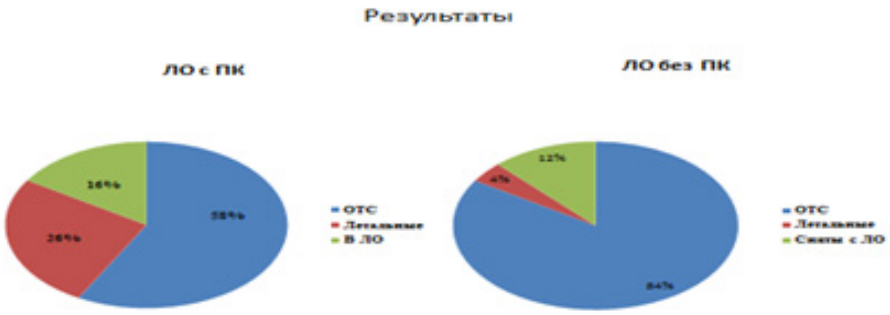

Рис. 11. В группе ЛО на ПК 14 (58%) больным выполнена ОТС, 6 (26%) пациентов умерли от ЛО, 4 (16%) пациентов продолжают находиться в ЛО на ОТС

В I группе оценка ФК сердечной недостаточности по NYHA до медикаментозной терапии составила: II ФК по NYHA - 25,2% пациентов, III ФК по NYHA - 50% пациентов, IV ФК по NYHA - 24,8% пациентов; при медикаментозной терапии: II ФК по NYHA - 11% пациентов, III ФК по NYHA - 57%, IV ФК по NYHA - 32%; после ОТС: I ФК по NYHA - 64,2%, II ФК по NYHA - 35,7%.

Во II группе оценка ФК сердечной недостаточности по NYHA до медикаментозной терапии: II ФК по NYHA - 40% пациентов, III ФК по NYHA - 52% пациентов, IV ФК по NYHA - 8% пациентов; при медикаментозной терапии: II ФК по NYHA - 38% пациентов, III ФК по NYHA - 55%, IV ФК по NYHA - 5%; после ОТС: I ФК по NYHA - 70%, II ФК по NYHA - 30%

Показатели тяжести состояния в исследуемых группах пациентов ХСН в ЛО до, во время медикаментозной терапии и после ОТС за недостаточностью кровообращения представлены на рис. 10.

Во II группе оценка тяжести состояния пациентов до, во время медикаментозной терапии с недостаточностью кровообращения. До терапии: НК IIA - у 71% пациентов, НК II B - у 29% пациентов; во время терапии НК ПА - у 75% пациентов, НК II B - у 25% пациентов; после ОТС: НК 0 – 100%, НК I-30%. Результаты исследавания представленны на рис. 11.

Распределение осложнений: острый криз отторжения - 5 (36%) пациентов; ПЖ недостаточность трансплантированного сердца - 1 (7%) больной, без осложнений - 8 (57%) пациентов. А в группе ЛО без ПК 21 пациенту выполнена прямая трансплантация сердца. 3 (12%) пациентов сняты с ЛО в связи с восстановлением миокарда, 1 (4%) больной умер в ЛО на терапии.

Выводы. Потенциальные реципиенты на трансплантацию сердца требуют тщательного и всестороннего обследования с целью выявления сопутствующей патологии и прогнозирования риска оперативного вмешательства и послеоперационных осложнений.

Своевременное направление пациентов на обследование и включение в лист ожидания является важнейшим фактором, поскольку период ожидания может занять длительное время.

На основании проведенного исследования разработаны и внедрены критерии отбора пациентов для включения в лист ожидания на трансплантацию сердца: ФВ ЛЖ - <20% (p<0,0001), давление заклинивания легочной артерии (ДЗЛА) - не более 35 мм рт. ст. (P<0,0001), пиковое потребление кислорода миокардом - <14 мл/кг/мин на фоне максимальной медикаментозной терапии (p<0,0001), ЛСС - <5 единиц за Wood (p<0,0001), ТПГ - до 15 мм рт. ст. (P<0,0001).

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SUMMARY

THE MAIN CRITERIA FOR SELECTING PATIENTS WHEN FORMING A WAITING LIST FOR HEART TRANSPLANTATION

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The aim of the study was to optimize the methods of selecting patients for inclusion in the «WAITING LIST» for heart transplantation on the basis of available international data and the introduction of selection criteria.

The results of the study are based on survey data and dynamic monitoring of 49 patients (median age 38 (16; 65) years, men 44 patients, women 5 patients) who were treated from 2008-2018 in the centers: Republican Scientific and Practical Center «Cardiology», Minsk, Republic of Belarus; in the Center of cardiac surgery on the basis of KL «Feofania» DUS, Kyiv, Ukraine. Patients were examined during the initial examination, after 3 months, 6 months and after 1 year.

The first group consisted of 24 patients with CHF who were on the waiting list for orthotopic heart transplantation, for circulatory support, median age 40.95 (18.0; 65.0) years, men - 23, women - 1; the second group consisted of 25 patients with CHF who were on the waiting list for orthotopic heart transplantation without circulatory support, median age 38.56 (17.0; 64.0) years, men -21, women-4; Scientific novelty of the obtained results.

Scientific novelty of the obtained results. For the first time in Ukraine, a road map has been developed and implemented and the dynamics of the movement of recipients who are in the "waiting list" for heart transplantation has been analyzed. Identified risk factors that affect the long-term outcomes and quality of life of patients with heart failure III-IV functional class according to the NYHA classification. Criteria for selection of patients for primary heart transplantation have been developed and implemented. For the first time in Ukraine, a "Waiting List" for a heart transplant has been formed.

Keywords: chronic heart failure, pulmonary hypertension, LVAD-therapy, BiVAD-therapy, orthotopic heart transplantation.

РЕЗЮМЕ

ОСНОВНЫЕ КРИТЕРИИ ОТБОРА ПАЦИЕНТОВ ПРИ ФОРМИРОВАНИИ ЛИСТА ОЖИДАНИЯ НА ТРАНСПЛАНТАЦИЮ СЕРДЦА

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Цель исследования - оптимизация критериев отбора пациентов для включения в лист ожидания на трансплантацию сердца на основании имеющихся международных данных и их внедрение в практику.

В исследование включено 49 больных (медиана возраста 38 (16; 65) лет, мужчин - 44, женщин - 5), которые находились на лечении в 2008-2018 гг. в Республиканском научно-практическом центре «Кардиология» (Минск, Республика Беларусь) и Центре кардиохирургии на базе Клинической больницы «Феофания» Государственного управления внутренними делами. Больные проходили обследование при поступлении и спустя 3, 6 месяцев и 1 год.

I группу составили 24 пациента с хронической сердечной недостаточностью (ХСН), которые находились на поддержке кровообращения в листе ожидания на ортотопическую трансплантацию сердца. Медиана возраста составила 40,95 (18; 65) г., мужчин было 23 и одна женщина.

II группу составили 25 пациентов с ХСН, находящихся в листе ожидания на ортотопическую трансплантацию сердца без поддержки кровообращения, медиана возраста -38,56 (17; 64) года, мужчин было 21, женщин - 4.

В Украине впервые разработана и внедрена дорожная карта и проанализирована динамика движения реципиентов, находящихся в листе ожидания на трансплантацию сердца. Установлены факторы риска, влияющие на отдаленные результаты и качество жизни пациентов с сердечной недостаточностью III-IV функционального класса по классификации NYHA. Разработаны и внедрены критерии отбора больных к первичной трансплантации сердца, а также составлен лист ожидания на трансплантацию сердца.

რეზიუმე

პაციენტების შერჩევის ძირითადი კრიტერიუმები გულის ტრანსპლანტაციის მოსაცდელი სიის ფორმირებისათვის

ო.ტანსკაია, ი.ოსტროვსკი, ე.კურლიანსკაია, ა. ვალენტიუკევიჩი, მ. კოლიადკო

კლინიკური საავადმყოფო "ფეოფანია", შინაგან საქმეთა სახელმწიფო სამმართველო, კიევი, უკრაინა; რესპუბლიკური სამეცნიერო-პრაქტიკული ცენტრი "კარდიოლოგია", მინსკი, ბელარუსის რესპუბლიკა

კვლევის მიზანს წარმოადგენდა გულის ტრანსპლანტაციისათვის მოსაცდელ სიაში პაციენტების ჩართვის კრიტერიუმების ოპტიმიზება არსებული საერთაშორისო მონაცემების საფუძველზე და მათი დანერგვა პრაქტიკაში.

კვლევაში ჩართული იყო 49 პაციენტი (ასაკის მედიანა – 38 (16; 65) წელი, მამაკაცი – 44, ქალი – 5), რომლებიც მკურნალობდნენ რესპუბლიკურ სამეცნიერო-პრაქტიკულ ცენტრში "კარდიოლოგია" (მინსკი, ბელარუსის რესპუბლიკა) და კარდიოქირურგიის ცენტრში კლინიკური საავადმყოფო "ფეოფანია"-ს ბაზაზე (უკრაინა). პაციენტებს გამოკვლევა ჩაუტარდა კლინიკაში შემოსვლისას, 3, 6 თვის და 1 წლის შემდეგ.

I ჯგუფი შეადგინა დამხმარე სისხლის მიმოქცევაზე მყოფმა 24 პაციენტმა გულის ქრონიკული უკმარისობით, რომლებიც იმყოფებოდნენ მოსაცდელ სიაში გულის ორთოტოპიულ ტრანსპლანტაციაზე. ასაკის მედიანამ შეადგინა 40,95 (18; 65) წელი, მამაკაცი – 23, ქალი – 1.

II ჯგუფი შეადგინა 25 პაციენტმა გულის ქრონიკული უკმარისობით, დამხმარე სისხლის მიმოქცევის გარეშე, რომლებიც იმყოფებოდნენ მოსაცდელ სიაში გულის ორთოტოპიულ ტრანსპლანტაციაზე. ასაკის მედიანამ შეადგინა 38,56 (17; 64) წელი, მამაკაცი – 21, ქალი – 4.

უკრაინაში პირველადაა შემუშავებული და დანერგილი საგზაო რუკა და გაანალიზებულია რეციპიენტების მოძრაობის დინამიკა, რომლებიც იმყოფებოდნენ მოსაცდელ სიაში გულის ტრანსპლანტაციაზე. დადგენილია გულის უკმარისობის III-IV ფუნქციური კლასის (NYHA-ს მიხედვით) მქონე პაციენტების შორეულ შედეგებსა და სიცოცხლის ხარისხზე მოქმედი რისკის ფაქტორები. შემუშავებული და დანერგილია პაციენტების შერჩევის კრიტერიუმები გულის პირველადი ტრანსპლანტაციისათვის, ასევე, შედგენილია მოსაცდელი სია გულის ტრანსპლანტაციისათვის.

COMPUTED TOMOGRAPHY IN DETECTING FEATURES OF CORONARY ATHEROSCLEROSIS IN DIFFERENT ETHNIC GROUPS OF KAZAKHSTAN POPULATION

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According to a National data of Kazakhstan, mortality from of cardiovascular diseases after the collapse of Soviet Union in early 1990's dramatically increased [1]. With the implementation of National programs since 2008 aimed at controlling modifiable risk factors and development of cardiac centers with catheterization labs and cardiac surgery units, the mortality rate has been drastically decreased by almost 30% [2]. In recent years cardiovascular centers of the country moving towards the prevention of heart events using technology and well-controlled prospective life-style modification studies [3]. Coronary computed tomography (CT) has been widely used in assessment of atherosclerosis burden by the means of quantification and detection of coronary artery calcium (CAC) score and level of stenosis [4]. Nonetheless, there are no works devoted to the assessment of CAC in an association with coronary events in Kazakhstan using CT scanning. According to large prospective studies, CAC score showed a relationship with the risk of future coronary events [5]. CAC score has been shown to be related to the severity of coronary artery disease by one of the biggest studies to date, Multi-Ethnic Study of Atherosclerosis (MESA) [6]. The major drawbacks of using CAC score as a predictor of risk along with the Framingham risk score has been jeopardized by the evidence that there are considerable differences in the extent of CAC among different ethnical groups [7]. Thus, the utmost interest lies in the assessment of structural variability of the coronary artery bed. Currently, no data available to address the prevalence and quantification of CAC score in ethnic groups among Kazakhstani population. It poses an important value to assess the possible ethnic-dependent variations of CAC score due to the fact that Kazakhstan is ethnically very diverse country. About 75% of population are represented by Kazakhs and other Central Asian nations (such as Uigurs, Uzbeks, Tatar's and other), while about 25% account for Russians and other European nationalities [8].

Coronary artery calcification (CAC) score has been proposed as a surrogate method to evaluate coronary atherosclerosis. Multiple studies worldwide defined CAC score and ethnicity association to be valuable in coronary event prognosis along with other established risk-factors. The objectives of this study were to determine the ethnic differences of CAC score in Kazakhstani population with stable chest pain and whether CAC is significantly associated with the traditional cardiovascular risk factors.

Material and methods. The study population included medical records of patients who were referred by physicians to undergo CT. Prior to CT scanning, all patients gave informed consents to utilize the collected medical data for research purposes. The study protocol has received an ethical approval from the local Hospital Research Ethics Committee.

935 patient medical records were collected between 2008 to 2018 from Medical Centre Hospital of President's Affairs Administration and National Research Cardiac Surgery Center (NRCS) in Astana, both provide medical care for patients across Kazakhstan. Patients with stable chest pain were included in the study. Patients with history of myocardial infarction, with cardiomyopathies, previously underwent coronary artery bypass graft (CABG), percutaneous coronary angioplasty or valve replacement procedures and those with chronic rheumatic heart diseases were not included in the study.

CT scanning was conducted on dual-source, Multislice CT scanner ("Somatom Definition AS 64 or Somatom Definition Flash, Siemens, Germany") with prospective cardiac synchronization and reconstruction with 0.6 mm slice thickness. The Agatston calcium score was quantified using commercially available software ("syngo Calcium Scoring, Siemens, Germany"), and calcification was defined as an area≥1 of density ≥130 Hounsfield Units (HU). The summary of all coronary lesion scores represented coronary artery calcification (CAC) score. CT coronary angiography (CTA) data were obtained with injection of contrast agent (Visipaque, 100 ml) intravenously (4 ml/sec). Stenosis level was assessed based on comparison of minimum lumen diameter (MLD) with non-affected arterial segment in closest proximity to the lesion.

Patients were categorized to Kazakh (66.9%), Russian (21.4%) and other (11.7%) ethnicities, which included Tatar, Ukrainian, German, Uzbek, Uygyr, Belarus, Kyrgyz and those who did not specified their ethnicities (3.1%). Additionally, demographic variables (age, gender, body mass index (BMI)) and CHD risk factors (maximally registered systolic (SBP) and diastolic blood pressure (DBP); hypertension, defined as having history of hypertension or SBP ≥ 140 mm of Hg or DBP ≥90 mm of Hg [9]; smoking status (smoker or non-smoker); alcohol use (whether a patient consumed any amount of alcohol drinks for the past year, yes or no); family history of CVD (whether a patient has a first or second-degree relative with CVD) [10]; diabetes mellitus, defined as having a history of diabetes mellitus or glucose ≥7 mmol/l or Hemoglobin A1c ≥ 6.5%; and plasma factors, such as, fibrinogen, creatinine, cholesterol level (total, low-density lipoprotein (LDL) and high-density lipoprotein(HDL)) and triglycerides) were collected from patient medical records. The type of chest pain was categorized to atypical, typical and non-anginal [18].

Continuous variables were summarized as means and standard deviations. When a continuous variable distribution was highly skewed, additionally, median and interquartile range (IQR) were included. To compare demographic characteristics and CHD risk factors' distributions among the ethnic groups, one-way ANOVA test or Kruskal-Wallis test, for categorical independent variables Pearson's chi-square test or Fisher's exact test were utilized.

The outcome variable, CAC score, has extremely right skewed distribution and frequent zero values. Given non-normality of CAC score, relationships between independent variables and continuous CAC score were assessed applying non-parametric tests (Kruskal-Wallis test, Spearman's correlation coefficient, Mann-Whitney U test). In addition, it was decided to dichotomize CAC score (any CAC score and none) to apply multivariate logistic regression analysis examining the association

between ethnicity and CAC score, adjusting for possible confounders and testing for an existence of interactions. After exclusion of all zero values, and log-transformation of the continuous CAC score, similarly, multivariate linear regression analysis was performed. In both modeling approaches, confounder selection was based on statistically significant (α=0.20) results from bivariate analyses and previous epidemiological studies [11]. In multivariate analyses, all statistically significant (α=0.05) and epidemiologically important covariates were left in the models to compare. All statistical analyses were performed using Stata 13 software [12].

Results and discussion. The study population consisted of 935 patients where average age among ethnic groups significantly differed (p<0.01); more than half patients in other group were older than 60 years (Table 1). There were statistically significant differences among ethnic groups by BMI (p=0.03), type of chest pain (p<0.01), use of statins (p=0.01) and blood glucose level (p<0.01). Russians had the lowest values of percent of statin users (38%) and glucose level (5.4±1.0). Kazakhs had the lowest proportion of obese people among three groups (42.0% vs 53.8% and 52.0% in Russian and others, respectively, p<0.03).

Table 1. Overall descriptive statistics of participants by ethnicity

Variables	Kazakh n=626	Russian n=200	Other n=109	p-value
Age, (yrs)	58.1 ± 11.0	59.6 ± 11.4	61.9 ± 9.2	<0.01*
Categorical Age (%)				
≤ 50	145 (23.2)	43 (21.5)	11 (10.2)	0.01**
51-60	206 (32.9)	63 (31.5)	33 (30.5)	
61-70	202 (32.3)	58 (29.0)	45 (41.7)	
> 70	73 (11.7)	36 (18.0)	19 (17.6)	
Female Male	197 (31.5) 429 (68,5)	78 (39.0) 122 (61)	39 (36.1)70 (64,2)	0.13** 0.15**
BMI Normal (%)	97 (17.5)	29 (15.6)	18 (17.6)	0.03**
BMI Overweight (%)	225 (40.5)	57 (30.6)	31 (30.4)	
BMI Obese (%)	233 (42.0)	100 (53.8)	53 (52.0)	
Systolic BP, mm of Hg	175.2±30.4	181.0±29.8	179.5±25.6	0.07*
Diastolic BP, mm of Hg	97.7±11.9	99.7±11.5	98.6±11.6	0.19*
Hypertension (%)	517 (82.8)	168 (84.4)	98 (89.9)	0.18**
Smoking (%)	119 (19.8)	51 (26.0)	29 (26.8)	0.08**
Alcohol use (%)	65 (10.8)	26 (13.3)	16 (14.8)	0.37**
Family History of CVD (%)	236 (39.5)	84 (42.9)	49 (46.2)	0.33**
Chest pain (%)				
Atypical	175 (29.6)	52 (26.2)	28 (26.2)	<0.01**
Typical	206 (34.8)	92 (46.2)	59 (55.1)	
Non-anginal	211 (35.6)	55 (27.6)	20 (18.7)	
Use of statins (%)	285 (50.3)	70 (38.0)	47 (45.2)	0.01**
Diabetes (%)	166 (26.7)	45 (22.6)	26 (23.8)	0.46**
HbA1C,%	6.9±2.1	5.9±1.2	6.0±1.8	0.06*
Glucose, mmol/l	5.9±1.8	5.4±1.0	5.8±1.7	<0.01*
Fibrinogen, g/l	3.0±0.9	3.0±0.9	3.1±1.0	0.38*
Creatinine, μmol/l	78.7±21.2	81.8±19.8	80.1±17.9	0.20*
Total Cholesterol, mmol/l	5.0±1.2	5.0±1.2	4.9±1.3	0.67*
LDL, mmol/l	3.3±1.1	3.3±1.1	3.3±1.1	0.78*
HDL, mmol/l	1.3±0.5	1.3±0.6	1.3±0.5	0.60*
Triglycerides, mmol/l	1.7±1.2	1.5±0.8	1.7±1.2	0.29*

Percentages are shown in parentheses. Continuous variables are presented as mean ± SD;
**- One-way ANOVA test or Kruskal-Wallis test when parametric assumptions were violated;*
*** - Pearson's chi-square test or Fisher's exact test*

CAC score, stenosis in coronary arteries and number of vessels with ≥50% stenosis were statistically significantly associated with ethnic descent of the patients (Table 2). Russians had, on average, the highest CAC scores in each of the coronary arteries, and one third of them presented with ≥ 50% stenosis of RCA. Kazakhs, on the other hand, had the lowest CAC scores in each of the coronary arteries and suffered less from stenosis of RCA (10.8%). Relatively, lower number of Kazakh patients (11.7%) presented with ≥50% stenosis of two or more vessels in comparison to Russians (16.0%).

Table 2. Characteristics of coronary CT findings by ethnicity

Variables	Kazakh n=626	Russian n=200	Other n=109	p-value
CAC (total)				
mean±SD	107.9±270.4	225.3±507.5	164.7±384.2	<0.01*
median (IQR)	7.5 (0-84.1)	17.3 (0-132.3)	28.4 (0-127.1)	
CAC in LAD				
mean±SD	48.8±118.1	95.0±210.3	71.8±155.8	0.08*
median (IQR)	0.9 (0-40.3)	3 (0-54.2)	9.8 (0-77.5)	
CAC in LCX				
mean±SD	16.4±49.6	42.2±149.4	36.6±84.8	<0.01*
median (IQR)	0 (0-4.8)	0 (0-13.2)	0 (0-25.8)	
CAC in RCA				
mean±SD	30.9±123.5	63.2±257.8	44.9±183.1	0.14*
median (IQR)	0 (0-4.4)	0 (0-11.0)	0 (0-9.0)	
CAC in LM				
mean±SD	8.2±47.0	23.6±88.4	19.4±65.7	<0.01*
median (IQR)	0 (0-0)	0 (0-6.1)	0 (0-1.2)	
Stenosis of LAD				
None or<50%	444 (73.6)	146 (74.5)	63 (60.0)	<0.01**
≥ 50%	159 (26.4)	50 (25.5)	42 (40.0)	
Stenosis of LCX				
None or<50%	518 (89.8)	174 (88.3)	86 (81.1)	0.04**
≥ 50%	59 (10.2)	23 (11.7)	20 (18.9)	
Stenosis of RCA				
None or<50%	518 (89.2)	135 (69.6)	70 (68.6)	<0.01**
≥ 50%	63 (10.8)	59 (30.4)	32 (31.4)	
Stenosis of LM				
None or<50%	511 (98.8)	149 (100)	76 (100)	0.51**
≥ 50%	6 (1.2)	0 (0)	0 (0)	
Number of vessels with ≥50% stenosis				
None	447 (71.4)	113 (56.5)	49 (44.9)	<0.01**
Only one	106 (16.9)	55 (27.5)	35 (32.1)	
Only two	40 (6.4)	19 (9.5)	16 (14.7)	
Three or LMD	33 (5.3)	13 (6.5)	9 (8.3)	
LAD plaque present				
non-calcified	46 (13.3)	7 (6.1)	6 (8.0)	0.24**
low-density, non-calcified	224 (64.7)	79 (68.7)	52 (69.3)	
calcified	76 (22.0)	29 (25.2)	17 (22.7)	
CX plaque present				
non-calcified	32 (14.0)	9 (8.7)	7 (10.6)	0.20**
low-density, non-calcified	117 (51.3)	63 (61.2)	43 (65.2)	
calcified	79 (34.7)	31 (30.1)	16 (24.2)	

RCA plaque present				
non-calcified	39 (15.2)	11 (10.9)	9 (15.0)	0.01**
low-density, non-calcified	146 (57.1)	75 (74.3)	43 (71.7)	
calcified	71 (27.7)	15 (14.8)	8 (13.3)	
LM plaque present				
non-calcified	9 (14.8)	2 (7.2)	1 (7.1)	0.65**
low-density, non-calcified	24 (39.3)	13 (46.4)	8 (57.2)	
calcified	28 (45.9)	13 (46.4)	5 (35.7)	

Percentages are shown in parentheses. Continuous variables are presented as mean ± SD and median (IQR) below;
** - Kruskal-Wallis test; ** - Pearson's chi-square test or Fisher's exact test*

CAC score was associated with age (p<0.001), gender (p<0.001), BMI (p<0.05), SBP (p<0.01), hypertension (p<0.001), use of statins (p<0.01), diabetes (p<0.001) and creatinine (p<0.001) (Table 3). There was a close association between patient age and CAC score (test for trend p<0.001). Unlike continuous CAC, dichotomous CAC score was associated with type of chest pain (p=0.04), glucose (p<0.01) and triglycerides (p<0.01).

According to Bivariate analysis CAC score was strongly associated with coronary CT findings (Table 4).

Table 3. Bivariate analysis for continuous and dichotomous CAC score (CAC score=0 and CAC score > 0) with demographic variables and CV risk factors

Variables	**Continuous CAC score**	**p-value**	**CAC score=0 (n=355)**	**CAC score>0 (n=574)**	**p-value**
Age					
≤50	24.9±80.9	<0.001*	133 (37.6)	65 (11.3)	<0.001**
51-60	109.3±364.8		115 (32.5)	186 (32.4)	
61-70	178.0±354.4		84 (23.7)	219 (38.2)	
More than 70	301.9±478.5		22 (6.2)	104 (18.1)	
Female	65.2±188.0	<0.001***	161 (45.6)	152 (26.5)	<0.001**
Male	178.2±405.3				
Kazakh	107.9±270.4	<0.01*	256 (72.1)	365 (63.6)	0.03**
Russian	225.3±507.5		64 (18.0)	135 (23.5)	
Other	164.7±384.2		35 (9.9)	74 (12.9)	
BMI					
Normal BMI	99.1±238.1	0.05*	70 (22.2)	73 (14.0)	<0.01**
Overweight	158.0±404.4		117 (37.2)	193 (37.0)	
Obese	140.4±343.5		128 (40.6)	256 (49.0)	
SBP	0.14	<0.001****	172.9±32.4	179.1±28.1	0.01***
DBP	0.07	0.06****	97.1±12.7	98.9±11.3	0.07***
Hypertension					
yes	156.4±363.9	<0.001***	266 (75.6)	512 (89.2)	<0.001**
no	55.1±263.7				
Smoking status					
yes	109.5±312.9	0.43***	78 (22.8)	121 (21.8)	0.71**
no	150.5±367.1				
Alcohol use					
yes	181.7±513.3	0.72***	38 (11.0)	69 (12.4)	0.52**
no	135.2±328.7				
Family history of CHD					
yes	114.8±275.1	0.41***	138 (40.3)	230 (41.7)	0.68**
no	158.9±402.8				

Chest pain					
Typical	177.3±436.0	0.21*	128 (37.8)	227 (41.1)	0.04**
Atypical	101.7±242.1		86 (25.4)	166 (30.0)	
Non-anginal	128.7±323.9		125 (36.9)	160 (28.9)	
Use of Statins					
yes	181.4±433.4	<0.01***	123 (38.8)	275 (51.8)	<0.001**
no	107.9±270.6				
Diabetes					
yes	158.8±315.2	<0.001***	66 (18.8)	169 (29.6)	<0.001**
no	133.8±364.2				
HbA1C	0.10	0.32****	6.4±1.5	6.7±2.1	0.65***
Glucose	0.06	0.09****	5.6±1.4	5.9±1.8	<0.01***
Fibrinogen	0.03	0.42****	2.98±0.8	3.08±1.0	0.15***
Creatinine	0.17	<0.001****	75.9±20.0	81.7±20.6	<0.001***
Total Cholesterol	0.04	0.23****	4.9±1.2	5.1±1.2	0.15***
LDL	-0.01	0.70****	3.3±1.1	3.3±1.0	0.90***
HDL	-0.06	0.07****	1.3±0.5	1.3±0.5	0.48***
Triglycerides	0.07	0.06****	1.5±0.8	1.7±1.3	<0.01***

Percentages are shown in parentheses. Continuous variables are presented as mean ± SD;
** - Kruskal-Wallis test; **Pearson's chi-square test or Fisher's exact test; *** - Two-sample t-test or Mann-Whitney U-test;*
***** - Spearman's correlation coefficient*

Table 4. Bivariate analysis for continuous and dichotomous CAC score (CAC score=0 and CAC score >0) with coronary CT variables

Variables	**Continuous CAC**	**p-value**	**CAC=0 (n=355)**	**CAC>0 (n=574)**	**p-value**
Stenosis of LAD					
None or<50%	53.4±169.6	<0.001***	329 (95.9)	322 (57.9)	<0.001**
≥ 50%	372.6±555.9		14 (4.1)	234 (42.1)	
Stenosis of LCX					
None or<50%	88.7±253.5	<0.001***	334 (98.8)	441 (82.1)	<0.001**
≥ 50%	566.5±657.5		4 (1.2)	96 (17.9)	
Stenosis of RCA					
None or<50%	80.3±242.2	<0.001***	331 (97.1)	389 (73.3)	<0.001**
≥ 50%	434.3±601.9		10 (2.9)	142 (26.7)	
Stenosis of LM					
None or<50%	134.3±359.8	<0.01***	310 (100)	422 (98.6)	0.04**
≥ 50%	394.6±377.3		0 (0)	6 (1.4)	
Number of vessels with stenosis ≥50%					
None	46.7±141.8	<0.001*	329 (92.7)	279 (48.6)	<0.001**
Only one	145.3±360.2		25 (7.0)	168 (29.3)	
Only two	443.4±464.9		0 (0)	73 (12.7)	
Three or LMD	744.9±740.5		1 (0.3)	54 (9.4)	
LAD plague present					
non-calcified	7.6±20.2	<0.001*	39 (86.7)	20 (4.1)	<0.001**
low-density, non-calcified	319.2±503.1		6 (13.3)	345 (70.8)	
calcified	68.1±142.2		0 (0)	122 (25.1)	
LCX plague present					

non-calcified	44.5±113.9	<0.001*	24 (85.7)	24 (6.6)	<0.001**
low-density, non-calcified	425.3±589.0		3 (10.7)	217 (59.4)	
calcified	129.1±231.6		1 (3.6)	124 (34.0)	
RCA plague present					
non-calcified	31.3±82.0	<0.001*	34 (87.2)	24 (6.4)	<0.001**
low-density, non-calcified	383.9±558.7		5 (12.8)	256 (68.5)	
calcified	108.5±178.3		0 (0)	94 (25.1)	
LM plague present					
non-calcified	101.6±159.1	0.02*	3 (100)	9 (9.1)	<0.01**
low-density, non-calcified	459.2±756.8		0 (0)	44 (44.4)	
calcified	230.2±363.6		0 (0)	46 (46.6)	

Percentages are shown in parentheses. Continuous variables are presented as mean ± SD;
**- Kruskal-Wallis test when parametric assumptions were violated; ** - Pearson's chi-square test or Fisher's exact test;*
**** - Mann-Whitney U test*

After adjusting for possible confounders in multivariate analyses, the odds of having positive CAC score increased by 48% (OR=1.48; 95% CI, 0.91 - 2.40) among Russians relatively to Kazakhs. Similarly, in the multivariate linear regression model, CAC score on average increased by 71.4% (p=0.03) between respective ethnic groups among patients with CAC score > 0. The odds of having positive CAC score were almost four times higher (OR=3.87, 95% CI, 2.57-5.84) among males relatively to females in logistic regression model adjusting for covariates. The odds of having positive CAC score and average percent of CAC score increased with older age groups (p<0.001). For example, average CAC was higher 103.5%, 271.0% and 645.6% among 51-60, 61-70 and >70 age groups, respectively, in reference to ≤50 age group. In multivariate analysis, relationships of CAC score with use of statins and SBP were positive.

Table 5. Multivariate linear regression coefficients for independent variables of CAC score among people with CAC score>0

Variable	Coefficient (95% CI) (log of CAC score)	Corresponding percentage change in CAC score (95% CI)	p-value
Kazakh	Reference	Reference	0.03
Russian	0.54 (0.13-0.95)	71.4% (1.4% to 141.3%)	
Other	0.26 (-0.23 to 0.74)	29.4% (-33.0% to 91.9%)	
Female	Reference	Reference	<0.001
Male	1.11 (0.73-1.50)	205.1% (89.6% to 320.6%)	
Age			
≤ 50	Reference	Reference	<0.001
51-60	0.71 (0.06 to 1.36)	103.5% (-28.4% to 235.4%)	
61-70	1.31 (0.67 to 1.96)	271.6% (32.4% to 510.8%)	
>70	2.01 (1.31-2.71)	645.6% (125.9% to 1165.2%)	
Family history of CVD			
no	Reference	Reference	0.02
yes	-0.41 (-0.75 to -0.07)	-33.6% (-56.3% to -10.9%)	
SBP, per 10 mm Hg	0.12 (0.05-0.18)	12.6% (5.3%-19.8%)	<0.001
Total cholesterol, per 10 mmol/l	0.22 (-1.17 to 1.61)	30.9% (-153.0% to 214.8%)	0.71
Use of statins			
no	Reference	Reference	0.02
yes	0.40 (0.06-0.74)	49.8% (-1.0% to 100.5%)	
Diabetes			
non-diabetic	Reference	Reference	0.54
diabetic	-0.11 (-0.48 to 0.25)	-10.7% (-43.3% to 21.9%)	
Intercept	-0.24 (-1.74 to 1.26)		0.76

Table 6. Odds ratio of CAC presence (CAC score>0) by independent variables in multivariate logistic regression

Variable		OR (95% CI)	p-value
Kazakh		1.00 (Reference)	0.28
Russian		1.48 (0.91-2.40)	
Other		1.16 (0.66-2.03)	
Female		1.00 (Reference)	<0.001
Male		3.87 (2.57-5.84)	
Age			
≤ 50		1.00 (Reference)	<0.001
51-60		2.61 (1.49-4.58)	
61-70		5.44 (3.01-9.84)	
>70		8.16 (3.95-16.89)	
Family history of CVD			
no		1.00 (Reference)	0.63
yes		1.14 (0.78-1.68)	
SBP*			
<150 mm Hg		1.00 (Reference)	0.03
150-179 mm Hg		1.04 (0.55-1.94)	
180-199 mm Hg		1.80 (0.93-3.50)	
≥200 mm Hg		1.90 (0.97-3.69)	
Total cholesterol*			
Below 5.2 mmol/l		1.00 (Reference)	0.59
5.2-6.1 mmol/l		0.96 (0.62-1.49)	
6.2 and above		1.26 (0.76-2.10)	
Use of statins			
no		1.00 (Reference)	0.56
yes		1.12 (0.76-1.64)	
Diabetes			
non-diabetic		1.00 (Reference)	0.04
diabetic		1.58 (1.02-2.44)	
Intercept		0.05 (0.01-0.18)	<0.001

** - Due to violation of the linearity assumption, SBP and total cholesterol were categorized to meet the assumption*

In this comparison between different ethnic groups in Kazakhstan with chest pain we have demonstrated that the burden of coronary atherosclerosis, assessed by calcium score, is greater among Russian and other ethnicities than among Kazakhs, even after adjustment for conventional risk factors. To our knowledge there were no previous studies on CAC score in Central Asian population and data about ethnic specificity of CV risk factors in this area are limited. Previous studies have shown that East Asians, including Chinese, Korean, and Japanese subjects, have lower CAD burden measured on CAC compared with Western subjects [13]. The Multi Ethnic Study of Atherosclerosis (MESA) also reported similar observations, whereby Chinese adults had a lower prevalence of CAC compared with Caucasian subjects [14]. Although there is a big difference in eating habit between Chinese and Kazakhs, seemingly lower CAC score is cross-race effect of Asians.

Many studies have shown that CAC score is strongly associated with age in different heterogeneous populations, geography and culture [15,16]. Higher CAC score among Russians could be attributed with higher prevalence of advanced aged patients in this group, 18% of patients were older than 70 years, in comparison with 11.7% and 17.6% in Kazakh and other ethnicity groups respectively.

Previous studies have shown that BP components have age-dependent roles in the prediction of CAC, and SBP and PP were independent predictors of the presence and quantity of coronary artery calcification in the ≥50 years of age group [17]. In our study, registered maximal systolic blood pressure among Russian population was higher compared to other groups. Systolic blood pressure was strongly associated with higher CAC score, both on Multivariate Linear and logistic regression analysis. Coronary calcium deposition may indicate higher degree of vascular calcification and, thus, impaired vascular compliance and higher blood pressure levels. But more investigations needed to clarify this mechanism.

Russian population had higher BMI and about 53% of Russians enrolled in the study were obese. Whereas, 41.9% of Kazakh population had BMI<30 kg/m^2. In bivariate analysis BMI showed positive correlation with CAC score, but multi-

variate linear or logistic regression analysis showed no impact of obesity on CAC score. In previous studies higher BMI is independently associated with increased risk of intermediate-term risk of myocardial infarction and abdominal obesity was an independent predictor of CAC progression [18], however, most studies evaluated the association between obesity and CAC in a Western population with conflicting results [19,20].

Several controversial findings were observed. One is character of chest pain which was mostly atypical or non-anginal among Kazakhs, when Russians and other ethnic groups had predominantly typical angina pectoris. One more interesting finding is, that Kazakhs had highest FPG level and multivariate analysis showed strong positive correlation between the presence of diabetes and CAC score. Also, plasma triglyceride levels were higher among Kazakhs compared with Russians and bivariate analysis shows positive correlation of triglyceride level and CAC score.

Several limitations of our study should be acknowledged. First, our study was performed at only two centers, those are located in one city and provides tertiary medical care, which makes it uncertain whether results will be likely generalizable to whole Kazakhstani population. Second, there were no standardized questionnaire to collect CV risk factors. Some of inconsistencies with previous studies results [reference], for example, family history of CVD in multivariate analyses, could be attributed to an absence of standardized approach in data collection. Additionally, there may be other confounders that were not included in adjustment, such as, physical activity, dietary habits and socioeconomic status. Third, this was a cross-sectional study with no prospective follow-up data on the patient management post CT scan and subsequent incidence of MI.

Conclusion. As compared with Russians, Kazakhs and other minorities, Kazakhs had significantly lower CAC score. The difference of the CV risk-factor profiles do not explain this finding. This study demonstrated that Russians have a higher atherosclerotic burden than Kazakhs and others, independent of risk-factor differences among patients with stable chest pain. Future longitudinal national and regional population-based studies are, however, warranted.

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SUMMARY

COMPUTED TOMOGRAPHY IN DETECTING FEATURES OF CORONARY ATHEROSCLEROSIS IN DIFFERENT ETHNIC GROUPS OF KAZAKHSTAN POPULATION

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The aim of the study was to identify the features of coronary lesions and to determine the correlation between the main risk factors for coronary artery disease according to the SCORE quality of life scale and the calcium index in MSCT in different age and ethnic groups in men and women living in Kazakhstan.

We retrospectively analyzed 935 case histories of patients undergoing MSCT to assess the condition of the coronary arteries. The patients were divided into three groups: Kazakhs (66.9%), Russians (21.4%) and other (11.7%) nationalities. There were statistically significant differences between ethnic groups in BMI ($p=0.03$), type of chest pain ($p<0.01$), statin use ($p=0.01$), and blood glucose ($p<0.01$). The study showed that the prevalence of coronary atherosclerosis is higher among Russians compared to Kazakhs, even after adjusting for traditional risk factors. In multivariate analysis, the calcium index values were significantly higher in the group of the Russian population by 48% (OR=1.48; 95% CI 0.91–2.40) than in the Kazakh population. In the course of the cross-sectional study, statistically significant differences in the nature of coronary lesions were revealed between ethnic groups, mainly males, living in the Republic.

Until now, such studies have not yet been conducted among the inhabitants of Kazakhstan, and data on the ethnic specificity of risk factors for cardiovascular diseases in this geographical region have not been sufficiently studied. Previous studies have shown that East Asians, including Chinese, Koreans, and Japanese, have a lower incidence of coronary artery disease as measured by CI compared to Europeans. A large MESA study also reported observations that study participants of Chinese nationality had a lower CI compared to Europeans. Despite significant differences in dietary habits and living in different climatic conditions between Asians of different countries, lower CI scores appear to be a racial trait of Asians, which was further confirmed by our study.

These results are undoubtedly representative, as patients from different regions of Kazakhstan were treated in two clinics of republican significance. In the future, it is necessary to conduct prospective studies with subsequent follow-up of patients after treatment and in identifying the causes of recurrent coronary events, as was done in the MESA study.

Keywords: atherosclerosis, coronary arteries, computed tomography, risk factor, inhabitants of Kazakhstan.

РЕЗЮМЕ

КОМПЬЮТЕРНАЯ ТОМОГРАФИЯ В ВЫЯВЛЕНИИ ОСОБЕННОСТЕЙ КОРОНАРНОГО АТЕРОСКЛЕРОЗА У ЖИТЕЛЕЙ РАЗЛИЧНЫХ ЭТНИЧЕСКИХ ГРУПП КАЗАХСТАНА

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Цель исследования - выявление особенностей поражения коронарного русла и определение корреляционной связи основных факторов риска ишемической болезни сердца с показателями кальциевого индекса в различных возрастных и этнических группах у мужчин и женщин, проживающих в Казахстане.

Ретроспективно проведен анализ 935 историй болезни пациентов, которым проведена мультиспиральная компьютерная томография (МСКТ) для оценки состояния коронарных артерий. Пациенты с учетом национальной принадлежности разделены на три группы: казахи (66,9%), русские (21,4%) и другие (11,7%) национальности. Между этническими группами выявлены статистически значимые различия по индексу массы тела ($p=0,03$), типу боли в груди ($p<0,01$), использованию статинов ($p=0,01$) и уровню глюкозы в крови ($p<0,01$). Исследование показало, что распространённость коронарного атеросклероза выше среди русской национальности в сравнении с казахской, даже после корректировки на традиционные факторы риска. Многофакторный анализ выявил, что показатели кальциевого индекса (КИ) превышают таковые группы русской популяции на 48% (OR=1,48; 95% ДИ 0,91–2,40) в сравнении в казахской. В ходе кросс-секционного исследования выявлены статистически значимые различия

по характеру поражения коронарного русла между этническими группами, преимущественно мужского пола, проживающими в Казахстане.

Авторы исследования считают необходимым проведение проспективных исследований с последующим динамическим наблюдением за пациентами после лечения с целью выявления причин повторных коронарных событий.

რეზიუმე

კომპიუტერული ტომოგრაფიის როლი კორონარული ათეროსკლეროზის თავისებურებების გამოვლენაში ყაზახეთში მცხოვრებ სხვადასხვა ეთნიკური ჯგუფის წარმომადგენლებში

[2,3]ე.ელშიბაევა, [1,2]ტ.დაუტოვი, [2]რ.რახიმჟანოვა, [4]მ.გუტბერლეტი, [2,3]დ.მარდენკიზი, [2]ჟ.კოჟახმეტოვა, [2,3]ა.სადუაკასოვა

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კვლევის მიზანს წარმოადგენდა კორონარული სისხლძარღვების დაზიანების თავისებურებების გამოვლენა და გულის იშემიური დაავადების ძირითადი რისკ-ფაქტორების კორელაციური კავშირის განსაზღვრა კალციუმის ინდექსთან ყაზახეთში მცხოვრებ სხვადასხვა ასაკის და სხვადასხვა ეთნიკური ჯგუფის წარმომადგენელ მამაკაცებსა და ქალებში.

რეტროსპექტულად გაანალიზებულია 935 პაციენტის ავადმყოფობის ისტორია, რომელთაც კორონარული არტერიების მდგომარეობის შეფასების მიზნით ჩაუტარდა მულტისპირალური კომპიუტერული ტომოგრაფია. პაციენტები, ეთნიკური კუთვნილების მიხედვით, დაიყო სამ ჯგუფად: ყაზახები (66,9%), რუსები (21,4%) და სხვა ეროვნების წარმომადგენლები (11,7%). ეთნიკურ ჯგუფებს შორის გამოვლინდა სტატისტიკურად მნიშვნელოვანი განსხვავება სხეულის მასის ინდექსის (p=0,03), გულმკერდის მიდამოში ტკივილის (p<0,01), სტატინების გამოყენების (p=0,01) და სისხლში გლუკოზის დონის (p<0,01) მიხედვით.

კვლევის შედეგებმა აჩვენა, რომ კორონარული ათეროსკლეროზის გავრცელება უფრო მაღალია რუსი ეროვნების მოსახლეობაში ყაზახებთან შედარებით, მათ შორის - ტრადიციული რისკ-ფაქტორების კორექციის შემდეგაც. მრავალფაქტორული ანალიზით გამოვლინდა, რომ რუსულ პოპულაციაში, ყაზახებთან შედარებით, 48%-ით უფრო მაღალია კალციუმის ინდექსის მაჩვენებლები (OR=1,48; 95%-იანი სანდოობის ინტერვალი 0,91–2,40). ქროს-სექციური კვლევით ყაზახეთში მცხოვრებ ეთნიკურ ჯგუფებს შორის, უპირატესად მამაკაცებში, გამოვლინდა სტატისტიკურად სარწმუნო განსხვავება კორონარული სისხლძარღვების დაზიანების ხარისხში.

განმეორებითი კორონარული შემთხვევების მიზეზების გამოსავლენად ავტორებს მიზანშეწონილად მიაჩნიათ პროსპექტული კვლევების ჩატარება მკურნალობის შემდეგ პაციენტებზე დაკვირვებით დინამიკაში.

TRANSFORMING GROWTH FACTOR AND ARTERIAL STIFFNESS IN PATIENTS WITH UNCONTROLLED ARTERIAL HYPERTENSION

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Arterial hypertension (AH) remains the leading cause of premature death and more than 200 million cases of disability worldwide [1]. In hypertensive patients who do not reach the target level of blood pressure (BP), accelerated remodeling of target organs develops [2], which increases the risk of cardiovascular complications. Arterial stiffness is a proven risk factor for cardiovascular complications in hypertension [3]. At the heart of vascular wall damage there are two processes - atherosclerosis and arteriosclerosis [4]. Numerous studies have shown a relationship between the degree of arterial stiffness and the presence of atherosclerotic lesions [5], but the data on the dependence of arteriosclerosis processes on classical risk factors for atherosclerosis is very contradictory [6,7]. In arteriosclerosis, media is affected: elastin degrades, collagen content increases, hyperplasia and hypertrophy of smooth muscle cells develop, and they are these processes that lead to an increase in arterial stiffness. It is known that transforming growth factor β1 (TGF-β1), a pleiotropic cytokine, enhances proliferation and growth of smooth muscle cells, as well as the accumulation of extracellular matrix [8], that is, the processes underlying target organ damage (TOD) in hypertension. It is a member of the superfamily of structurally related proteins, which includes at least 40 proteins [9]. Some

studies have identified the relationship of TGF-β1 with myocardial [10] and renal [11] remodeling in hypertension. The effect of TGF-β1 on (pulse wave velocity) PWV has been studied and hypersecretion of TGF-β1 has been revealed in violation of the elastic properties of arteries in hypertensive patients [11, 12]. However, this study did not research the relationship between the level of TGF-β1 and the cardio-ankle vascular index (CAVI). Today, it is the CAVI index that is the most promising marker of vascular lesions [13]. Its advantage is the absence of dependence on the blood pressure level at the time of measurement, in contrast to PWV [14]. Moreover, patients with controlled and uncontrolled hypertension were not analyzed. The study of the influence of new markers of arteriosclerosis and atherosclerosis on the remodeling of the vascular wall seems to be very relevant.

The aim of our study was to identify the relationship between TGF-β1, arterial stiffness, and target organ damage in patients with uncontrolled arterial hypertension (UAH).

Material and methods. The study included 140 patients, including 80 patients with controlled hypertension (CAH) and 30 with uncontrolled hypertension (UAH). 30 patients made up the control group. The CAH group included patients receiving continuous antihypertensive therapy with target blood pressure (BP) values for at least 1 year. The UAH group included patients who did not receive sufficient antihypertensive therapy or did not adhere to it, as a result of which they did not reach the target BP values. The control group consisted of healthy individuals. The criteria for the inclusion of patients in the study were the age of patients from 40 to 70 years old with a verified diagnosis of essential arterial hypertension. Criteria for exclusion from the study: refusal of the patient from further participation in the study, secondary hypertension, chronic heart failure stages II-III, atrial fibrillation, diabetes mellitus, chronic kidney disease with decreased glomerular filtration rate (GFR <60 ml/min/1,73 m^2; according to the CKD-EPI formula), stenosis of the brachiocephalic arteries (BCA) more than 50% according to ultrasonography, acute inflammatory diseases and/or exacerbations of chronic inflammatory diseases of the endocrine, respiratory, urinary systems, skin lesions in the area of overlapping cuffs, pathology of the venous and arterial channels, diseases of the central nervous system, taking psychotropic drugs, acute infectious diseases, malignant neoplasms. The study was conducted in accordance with the Declaration of Helsinki on Human Rights. All patients gave informed consent to participate in the study. The study design is shown in Fig. 1.

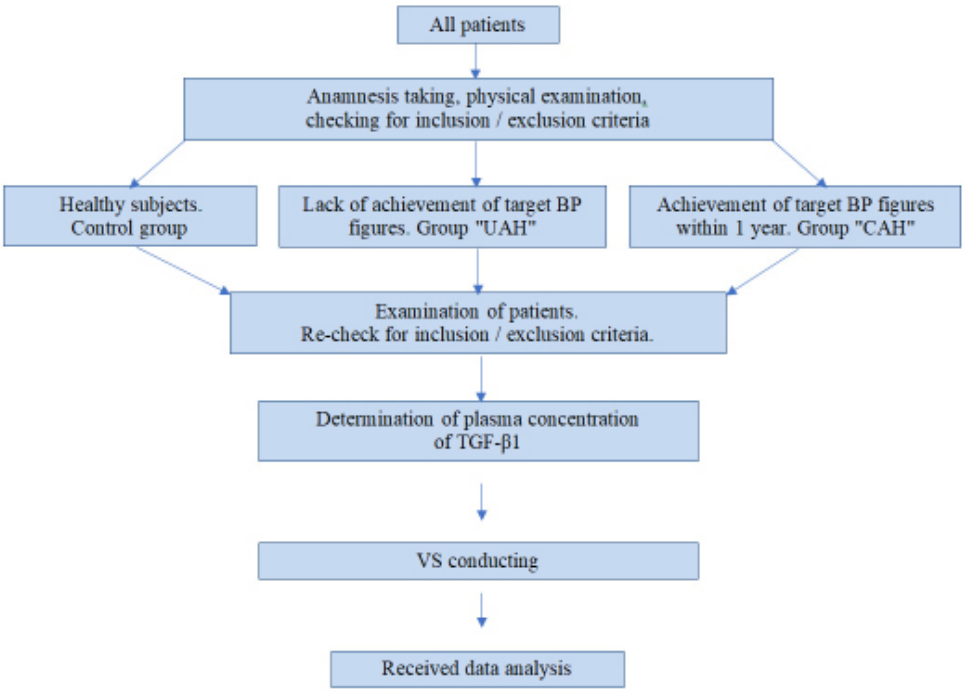

Fig. 1. The study design

All patients underwent a general clinical examination, including taking anamnesis, physical examination, laboratory and instrumental diagnostic methods. Doppler ultrasound (USG) with an assessment of the intima-media thickness (IMT) and determination of the degree of common carotid artery (CCA) stenosis was performed using the LOGIQ F6 apparatus (GE Healthcare, USA). The study of the diurnal dynamics of blood pressure was carried out with a BPLab device (LLC "Petr Telegin", Russia) according to the standard method. Determination of the arterial stiffness was performed using the cardio-ankle vascular index (CAVI) using the VaSera 1500-N apparatus (FukudaDenshi, Japan, 2012) by volumetric sphygmography (VS). The device non-invasively measures blood pressure on the upper and lower extremities with simultaneous recording of ECG, phonocardiogram (PCG), pulse waves of 4 extremities. The study was carried out in the supine position. Exercise and smoking were eliminated 20 minutes before the start. The cardio-ankle vascular index (CAVI), systolic and diastolic blood pressure in 4 limbs were determined. Determination of the plasma concentration of TGF-β1 was performed using the Human TGF-β1 Platinum ELISA BMS249 / 4 by ELISA. The results were processed using the STATISTICA 10.0 software package (StatSoftStatistica v10.0). The type of distribution of quantitative traits was analyzed using Shapiro-Wilk's W and Kolmogorov-Smirnov tests. When describing quantitative indicators with an abnormal distribution, medians with an interquantile range were used. Correlations were calculated by the parametric Pearson method, as well as by the nonparametric – Spearman method. When comparing

groups to measure the level of significance of differences, Student's T-test and Mann-Whitney U-test were used. The level of statistical significance was taken as p<0,05.

Results and discussion. The average age of the patients was 55 [49; 63] years old for the CAH group, 58 [56; 65] for the UAH group and 56,5 [48; 64] for the control group. All subjects were comparable in terms of sex, age, duration of AH (Table 1).

Drug therapy in the CAH group included angiotensin-converting enzyme inhibitors/angiotensin II receptor blockers - 58,1%, selective beta-blockers - 39%, thiazide/thiazide-like diuretics - 31%, calcium antagonists - 17%, other drugs - 0,8%. Antihypertensive therapy was carried out as monotherapy in 33% of patients, combined two-component therapy in 40%, three-component therapy in 25%, and four-component therapy in 3% of patients.

The TGF-β1 level was maximal in the UAH group and amounted to 22,6 [20,6; 25,6] ng/ml, in the CAH group it was 19,2 [17,2; 24,7] ng/ml. The lowest TGF-β1 value was found in the control group, 17,4 [11,8; 19,3] ng/ml (Fig. 2).

Table 1. Clinical characteristics of patients

Index	Group I (CAH) n=80	Group II (UAH) n=30	Group III (control group) n=30	p
Age, years	55[49;63]	58[56;65]	56,5[48;64]	p I,II,III>0,05
Sex, m/f, %	35/65	33/67	32/68	p I,II,III>0,05
BMI, kg/m2	27[24;32]	30,4[27;32,5]	23,4[20,8;26,7]	p I,II,III<0,05
Smoking, yes/no (pers.(%))	32(39%)/48(61%)	9(31%)/21(69%)	9(30%)/21(70%)	p I, II <0,05 p I, III <0,05 p II,III >0,05
Duration of hypertension, years	7 [4;11]	9[5;12]	-	p I,II>0,05
AH degree, 1 2 3, (pers. (%))	- 49 (38%) 31(62%)	- 11 (36%) 19 (64%)	-	p I,II>0,05
Myocardial mass, g	165[139;234]	210[165;269]	112[92;112]	p I,II,III<0,05
IMT, mm	1[0,9;1]	1[0,9;1]	0,8[0,7;0,9]	p I ,II>0,05 p I ,III<0,05 p II,III <0,05
Creatinine, μmol/L	93 [89;106]	94 [77;104]	79[75;82]	p I, II, >0,05 p I, III < 0,05 p II, III <0,05

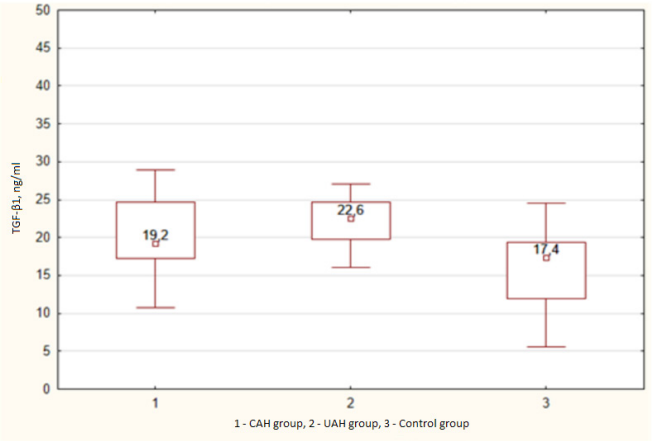

Fig. 2. TGF-β1 levels in groups

According to VS data, the maximum and minimum values of the CAVI indices were observed in the UAH group 9,2 [8,5; 9,9] and the control group 7 [6,5; 7,5], respectively (p <0,05), in the CAH group there was an intermediate value of the CAVI index of 7,8 [7,0; 8,5] (p <0,05). The correlation analysis revealed a significant relationship between the TGF-β1 level and CAVI for patients with hypertension (CAH r=0,777; UAH r=0,753; p<0,05) (Figs. 3 and 4). In the control group, no such relationship was found.

There was a significant difference in the levels of TGF-β1 depending on the stage of hypertension in CAH patients (p <0,05) (Fig. 5). In patients of the UAH group, there were no significant differences in the level of TGF-β1 at different stages.

According to USG CCA data, there was no significant difference in IMT between the CAH and UAH groups (p>0,05), and in both cases its mean value was higher than normal (>0,9 mm); however, the IMT value in the control group was significantly lower than in the CAH and UAH groups (p<0,05). Correlation analysis revealed the relationship between TGF-β1 and IMT only in the group of patients with hypertension (CAH r=0,509; UAH r=0,624; p<0,05). The relationship with the degree of CCA stenosis was not detected (p>0,05).

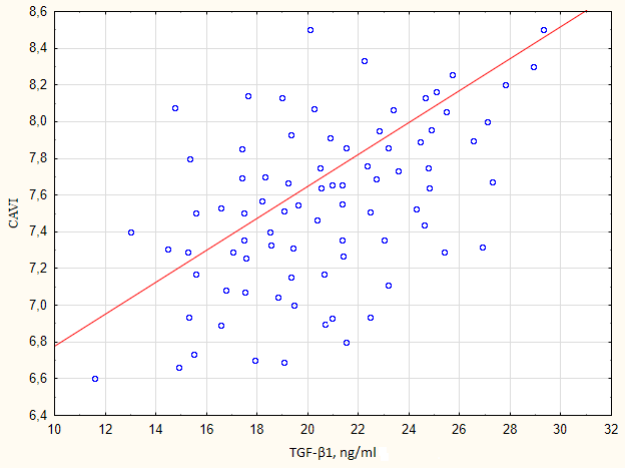

Fig. 3. Relationship between CAVI and TGF-β1 in the CAH group

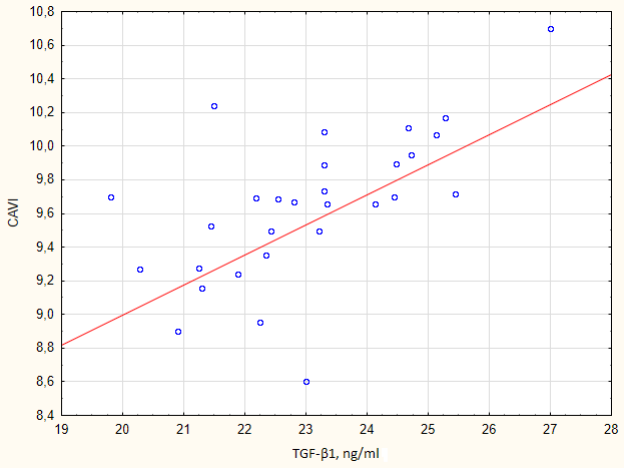

Fig. 4. Relationship between CAVI and TGF-β1 in the UAH group

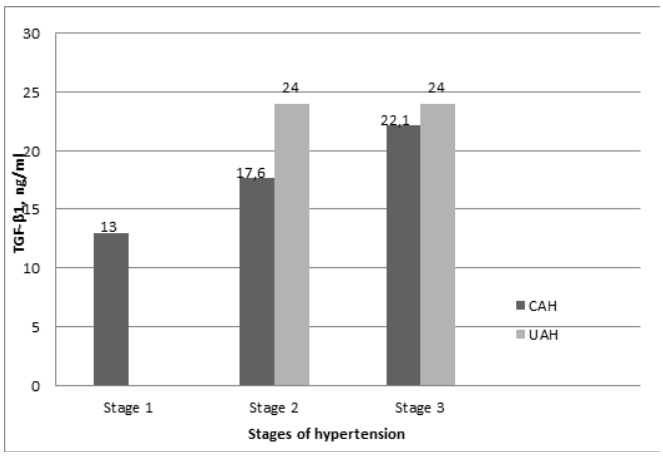

Fig. 5. Median TGF-β1 value depending on the stage of hypertension in the CAH and UAH groups

Table 2. Results of the regression model. One-way analysis

Index	Beta	Standard error	T-value	p
Myocardial mass, g	0,53	0,21	2,54	0,02
CAVI	0,70	0,23	3,05	0,009
Creatinine μmol/L	0,35	0,14	1,13	0,02

According to the results of the one-way regression analysis, it was revealed that in hypertensive patients with a TGF-β1 value, the most significantly correlated are: myocardial mass (MM) ($p<0,05$), CAVI ($p<0,05$) and creatinine level ($p<0,05$), Table 2.

The results of our study showed that the level of TGF-β1 was significantly higher in the group of UAH patients compared with CAH patients. The lowest TGF-β1 level was observed in patients without hypertension. Formerly, Derhaschnig U. [15] found an increase in TGF-β1 in patients with hypertension, in contrast to normotensive patients. Similar results were obtained by B. Li [16], in whose studies the effect of polymorphism of genes encoding TGF-β1 on blood pressure was also studied, while analysis depending on the achievement of target blood pressure values was not performed in this study. There is an opinion that an increase in the level of TGF-β1 against the background of hypertension may be associated with an increase in shear stress or an increase in the level of angiotensin II [17]. In our study, the highest TGF-β1 values were also observed in patients with more severe stages of the disease in the CAH group, in contrast to the UAH group, where there was no significant difference in TGF-β1 levels depending on the stage. An uncontrolled course of hypertension contributes to a significant increase in the pathological cytokine TGF-β1 at an earlier stage of the disease. Inevitable changes with prolonged increase in blood pressure affect the vascular wall. In the process of vascular remodeling, atherosclerosis develops — changes in the intima of the vessels and the development of arteriosclerosis — changes in the media of the vessels [18]. The positive correlation of TGF-β1 with IMT suggests its connection with changes in the vascular media. Probably due to the intensification of the accumulation of extracellular matrix and hypertrophy of smooth muscle cells in the middle layer of blood vessels, IMT in all patients with hypertension [19]. Progression of arteriosclerosis leads to an increase of the arterial stiffness - an integral marker of cardiovascular disease [20]. In our study, the maximum CAVI value was observed in patients with UAH, intermediate - in CAH, and minimum - in the control group. Probably, TGF-β1, participating in the processes of arteriosclerosis, contributes to

an increase of the arterial stiffness in patients with hypertension and, to a greater extent, in patients with uncontrolled hypertension. In the available literature, we did not find such studies examining the effect of TGF-β1 on the CAVI index. There are separate studies in the course of which the effect of TGF-β1 level on the of the arterial stiffness is investigated in mouse models by measuring PWV [21]. In general, the relationship between TGF-β1 and TOD is not limited to the vascular wall. According to the results of regression analysis in our study, a relationship between TGF-β1 and (MM) and creatinine level was revealed, which reflects changes in other AH target organs - the heart and kidneys. Thus, a significant increase in the level of TGF-β1 in patients with hypertension, especially uncontrolled hypertension, as well as the revealed relationship with CAVI, makes it possible to use TGF-β1 as a probable predictor of vascular wall damage in patients with hypertension.

Conclusion. In patients in the UAH group, there was an increase in the concentration of TGF-β1 and an increase of the arterial stiffnes in comparison with patients in the CAH group and the control group. The revealed relationship between TGF-β1 and arterial stiffness indices suggests a significant role of TGF-β1 in the development of arteriosclerosis in hypertension.

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SUMMARY

TRANSFORMING GROWTH FACTOR AND ARTERIAL STIFFNESS IN PATIENTS WITH UNCONTROLLED ARTERIAL HYPERTENSION

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The aim of our study was to identify the relationship between TGF-β1, arterial stiffness, and target organ damage in patients with uncontrolled arterial hypertension (UAH).

The study included 140 patients with hypertension: 30 people with uncontrolled hypertension (UAH), 80 people with controlled hypertension (CAH) and 30 people in the control group. All patients underwent determination of arterial stiffness using cardio-ankle vascular index (CAVI) and TGF-β1 level.

The TGF-β1 level was 19,2 [17,2; 24,7] ng/ml in the CAH group. In the UAH group – 22,6 [20,6; 25,6] ng/ml. In the control group - 17.4 [11,8; 19,3] ng/ml. The maximum and minimum values of the CAVI indices were observed in the group UAH 9,2 [8,5; 9,9] and control group 7 [6,5; 7,5], respectively (p<0,05), in the CAH group there was an intermediate value of the CAVI index 7.8 [7,0; 8,5] (p<0,05). The correlation analysis revealed a significant relationship between the TGF-β1 level and CAVI for patients with hypertension (CAG r=0,777; NAG r=0,753; p<0.05). There was a significant difference in the TGF-β1 levels depending on the stage of hypertension in patients CAH (p <0,05). According to the results of the one-way regression analysis, it was revealed that in hypertensive patients with a TGF-β1 value, the most significant interactions are between myocardial mass (MM) (p<0,05), CAVI (p<0,05) and creatinine (p<0,05).

An increase in the concentration of TGF-β1 and an increase of arterial stiffness were revealed in patients of the UAH group in comparison with the other groups. The relationship between TGF-β1 and arterial stiffness was also found.

Keywords: transforming growth factor β1, CAVI, arterial hypertension, target organ damage, arterial stiffness.

РЕЗЮМЕ

ТРАНСФОРМИРУЮЩИЙ ФАКТОР РОСТА И ЖЕСТКОСТЬ СОСУДИСТОЙ СТЕНКИ У ПАЦИЕНТОВ С НЕКОНТРОЛИРУЕМЫМ ТЕЧЕНИЕМ АРТЕРИАЛЬНОЙ ГИПЕРТЕНЗИИ

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Целью исследования явилось определить взаимосвязь между TGF-β1, жесткостью сосудистой стенки и поражением органов-мишеней у пациентов с неконтролируемым течением артериальной гипертензии.

В исследование включено 140 пациентов: 30 больных с неконтролируемым течением АГ (НАГ), 80 – с контролируемым течением АГ (КАГ) и 30 - группа контроля. Группу контроля составили здоровые исследуемые. У пациентов определена жесткость сосудистой стенки при помощи сердечно-лодыжечного сосудистого индекса (CAVI) и уровня TGF-β1.

Уровень TGF-β1 составил 19,2 [17,2;24,7] нг/мл в группе КАГ. В группе НАГ - 22,6 [20,6;25,6] нг/мл, в группе контроля - 17,4 [11,8;19,3] нг/мл. Максимальные и минимальные значения индексов CAVI наблюдались в группе НАГ - 9,2 [8,5;9,9] и группе контроля - 7 [6,5;7,5] (p<0,05), в группе КАГ отмечалось промежуточное значение индекса CAVI - 7,8 [7,0;8,5] (p<0,05). В ходе корреляционного анализа выявлена достоверная связь уровня TGF-β1 с CAVI у пациентов с АГ (КАГ r=0,777; НАГ r=0,753; p<0,05). Наблюдалась значимая разница в уровнях TGF-β1 в зависимости от стадии АГ у пациентов с КАГ (p<0,05). По результатам проведенного однофакторного регрессионного анализа выявлено, что у больных АГ с величиной TGF-β1 наиболее достоверно взаимодействуют: масса миокарда (p<0,05), CAVI (p <0,05) и уровень креатинина (p<0,05).

Выявлено повышение концентрации TGF-β1 и увеличение жесткости сосудистой стенки у пациентов группы НАГ в сравнении с остальными группами. Обнаружена взаимосвязь между показателями TGF-β1 и жесткости сосудистой стенки.

რეზიუმე

ზრდის მატრანსფორმირებელი ფაქტორი და სისხლძარღვის კედლის სიმყარე პაციენტებში არტერიული ჰიპერტენზიის არაკონტროლირებადი მიმდინარეობით

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[1]მოსკოვის ი.სეჩენოვის სახ. პირველი სახელმწიფო სამედიცინო უნივერსიტეტი, საფაკულტეტო თერაპიის №2 კათედრა; ნ.სკლიფოსოვსკის კლინიკური მედიცინის ინსტიტუტი; [2]დამატებითი განათლების ავტონომიური არაკომერციული ორგანიზაცია "Intenational Travel and Education Company (ITEC) უცხო ენების სკოლა"; მოსკოვი, რუსეთი

კვლევის მიზანს წარმოადგენდა ურთიერთკავშირის განსაზღვრა ზრდის მატრანსფორმირებელ ფაქტორს (TGF-β1), სისხლძარღვის კედლის სიმყარესა და სამიზნე ორგანოების დაზიანებას შორის პაციენტებში არტერიული ჰიპერტენზიის არაკონტროლირებადი მიმდინარეობით.

კვლევაში ჩართული იყო 140 პაციენტი: 30 – არაკონტროლირებადი არტერიული ჰიპერტენზიით, 80 – არტერიული ჰიპერტენზიის კონტროლირებადადი მიმდინარეობით, 30 – საკონტროლო ჯგუფი. საკონტროლო ჯგუფი შედგებოდა ჯანმრთელი პირებისგან. პაციენტებში გულ-კოჭის სისხლძარღვოვანი ინდექსის (CAVI) და TGF-β1-ის დონის მიხედვით განისაზღვრა სისხლძარღვის კედლის სიმყარე.

TGF-β1-ის დონემ კონტროლირებადი არტერიული ჰიპერტენზიის ჯგუფში შეადგინა 19,2 [17,2;24,7] ნგ/მლ, არაკონტროლირებადი არტერიული ჰიპერტენზიის ჯგუფში – 22,6 [20,6;25,6] ნგ/მლ, საკონტროლო ჯგუფში – 17,4 [11,8;19,3] ნგ/მლ. CAVI ინდექსის მაქსიმალური და მინიმალური მაჩვენებლები აღინიშნა არაკონტროლირებადი არტერიული ჰიპერტენზიის ჯგუფში – 9,2 [8,5;9,9] და საკონტროლო ჯგუფში – 7 [6,5;7,5] (p<0,05). კონტროლირებადი არტერიული ჰიპერტენზიის ჯგუფში აღინიშნა CAVI-ის საშუალო მაჩვენებელი – 7,8 [7,0;8,5] (p<0,05). კორელაციური ანალიზით გამოვლინდა TGF-β1-ის დონის სარწმუნო კავშირი CAVI-თან პაციენტებში არტერიული ჰიპერტენზიით (კონტროლირებადი არტერიული ჰიპერტენზიის ჯგუფში – r=0,777; არაკონტროლირებადი არტერიული ჰიპერტენზიის ჯგუფში – r=0,753; p<0,05). აღინიშნა TGF-β1-ის დონის მნიშვნელოვანი განსხვავება არტერიული ჰიპერტენზიის სტადიაზე დამოკიდებულებით კონტროლირებადი არტერიული ჰიპერტენზიის ჯგუფში (p<0,05). ჩატარებული ერთფაქტორიანი რეგრესიული ანალიზის

შედეგების მიხედვით გამოვლინდა, რომ პაციენტებში არტერიული ჰიპერტენზიით TGF-β1-თან ყველაზე სარწმუნოდ ურთიერთქმედებენ: მიოკარდიუმის მასა (p<0,05), CAVI (p<0,05) და კრეატინინის დონე (p<0,05).

დადგენილია TGF-β1-ის კონცენტრაციის და სისხლძარღვთა კედლის სიმყარის მატება პაციენტებში არაკონტროლირებადი არტერიული ჰიპერტენზიით, სხვა ჯგუფებთან შედარებით. აღმოჩენილია ურთიერთკავშირი TGF-β1-ის მაჩვენებლებსა და სისხლძარღვთა კედლის სიმყარეს შორის.

SENSITIVITY TO MECHANICAL PAIN BASED ON SATIETY LEVELS IN WOMEN

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According to the definition of the International Association for the Study of Pain, pain is subjective and emotional experience related to actual or potential tissue damage [24,27]. Pain is an important protective mechanism, as unpleasant sensation is coupled with urgency to cease irritating factor's action. Recently, gender-associated differences in perception of pain have been a subject of a number of studies and they revealed, that women tend to have a higher persensitivity and lower threshold to pain [1-3]. Sex differences are caused by several reasons, including biological, psychosocial and cultural factors [25,28]. For the studies of individual characteristics of pain perception, the system of pain modulation plays an important role; systemic research data suggests that inhibitory control of diffuse pain is weaker in women compared to men [21], and this is accentuated by the effect of sex hormones on central and peripheral nociceptive system [10,11] and specificity of endogenous opioid system receptor (Miu- and Kappa-) distribution in males and females [19]. In addition, the following psychosocial factors that contribute to increased pain sensitivity in women have been identified [19]: hypervigilance, higher body awareness, more attentive monitoring of bodily signals, higher prevalence of anxiety and depression, altering serotonin levels in the body. The idea of gender-associated differences in pain perception is supported by data from several studies [21,27]. In particular, cerebral activation induced by noxious stimuli was examined using positron-emission tomography and results indicated that 50°C thermal stimulus was evaluated as painful by more women than men (P<0.01) [8].

In the research of nociception characteristics and sex differences, the importance of ovarian-menstrual cycle should be taken into consideration, as the sex hormones act as neuroactive steroids [22]. On the one hand estrogen and progesterone are significant for analgesia [7,13], on the other hand – attention should be paid to antinociceptive effects of aforementioned hormones. One study has revealed that estrogen modulates pain during migraines, temporo-mandibular disorder and arthritis [18].

The recent data have shown that in addition to biological and psychosocial mechanisms, individual pain perception can be altered by satiety level as well [30]. Some studies confirm that there is a relationship between being fed by sucrose and hyperalgesia in animals [11], while others show that ketogenic diet can be associated with decreased pain sensitivity to thermal stimuli [20]. It can be argued that the effect of satiety level on pain perception is mediated by gastrointestinal hormones and endogenous opioids. Pharmacological stimulation of kappa-opioid receptors decreases stress and promotes analgesia. Accordingly, these changes can be inhibited by applying antagonists. Analysis of intraduodenal content postprandially identifies the impact of food on a person's mood and behaviour [26]. When food is present in stomach and intestines, several polypeptide hormones are released into the lumen and in the blood stream. The nature of secreted neurotransmitters depends on autonomic nervous system and composition of the food. Protein induces gastrin release in the antrum of the stomach, while cholecystokinin release is being activated by fat content. The synthesis of insulin depends on carbohydrates and is mediated by incretin peptides. Both cholecystokinin and insulin induce sleepiness postprandially in humans and in animals [12]. The mechanism of this relationship is not fully established, but it is proposed that the vagus nerve afferentation plays an important role – it transmits the signals to hypothalamus, which, in turn, stimulates oxytocin secretion. Hypothalamus, particularly ventromedial nucleus, is an important structure for pain modulation [6]. Oxytocin is associated with sedation and it can also be associated with cholecystokinin-related satiety. These alterations cause general relaxation, diminish anxiety and, in turn, decrease sensitivity to pain [15]. This idea is confirmed by the experiment on rats, which identified the influence of oxytocin on decreasing stress and anxiety [17]. Studies suggest that after eating, cholecystokinin causes different behavioral responses (satiety, tranquilization, sedation), but the exact pathway of pain alteration is not established. According to some pharmacological experiments, cholecystokinin worsens pain in humans and in rats [19], while other studies indicate to cholecystokinin-mediated analgesia.

In order to investigate, whether food could reduce pain perception or not, a study has been conducted, which aimed to show the gender related difference in pain perception during starvation, primary and secondary satiety states [30]. Although, due to limited data, objective relationship between pain and food intake could not be established and evaluated [30].

Our study aims to assess pain perception induced by mechanical experimental irritation in women during different satiety levels in follicular phase of ovarian-menstrual cycle.

Material and methods. The sample of the study was comprised of volunteer students aged 18-23 (women, mean age 19,5, standard deviation 2,9).

The main selection criterium for participants was their health state; Those without chronic pain, excess body weight (assessed by BMI), cardiovascular, respiratory, endocrine, etc. disorders were selected for participation in the study. Prior to the start of the study, participants were given information about their rights – they could refuse taking part in the study at any stage. Accordingly, written informed consents were obtained from every participant. All procedures and protocol of the study are approved by Tbilisi State Medical University Biomedical Research Committee. The study was conducted in compliance with all require-

Table. Mechanical Pain Threshold and Individual Resistance Threshold to Mechanical Pain: primary satiety and physiological starvation

	Physiological Starvation State	**Sensory-Motor Satiety State**
Mechanical Pressure Threshold	98±2,9 kPa	120±3,0 kPa
Mechanical Pain Threshold	297±1,98 kPa	350±2,6 kPa
Tolerance Threshold to Mechanical Pain	459±2,9 kPa	610±3,9 kPa

ments and regulations of International Pain Association for biomedical observation and experiments.

Ovarian-menstrual cycle of the women participating in the research were evaluated using the questionnaires. All the studies were performed in the follicular phase of menstrual cycle (7-11 days of the cycle).

At the first stage, study was conducted in starvation state – after 10-12 hours after the last meal, the second stage – in primary, sensory-motor satiety 20-30 minutes after intake of a mixed meal.

Every participant has been offered to take standard, mixed meal (including proteins, fats, carbohydrates).

The study was performed in isolated, sound-proof space. The proband (subject under observation/study) was placed in a comfortable armchair. The duration of the study was approximately 1-1.5 hours. The height, weight, blood pressure, body mass index (BMI) and other measurements were registered prior to the commencement of study.

Mechanical pain sensitivity was evaluated using computerized algometer - *AlgoMed (Medoc, Ltd, Ramat Yishai, Israel),* which was delivering mechanical stimuli to the participants; Meanwhile, mechanical pressure threshold, mechanical pain threshold and pain tolerance threshold were determined. A flat probe of algometer (surface area of 1 cm^2), was applied to the participant's palm delivering steadily increasing and quantifiable pressure at a rate of 30kPa/sec. The quantitative assessment of the parameters was automatically performed by pressing the remote control button.

Mechanical stimuli were delivered in the following sequence: 4 trials of mechanical pressure threshold (MPrTh), 4 trials of mechanical pain threshold (MPTh) and 4 trials of pain tolerance (MPT). In order to prevent sensitization/habituation of skin receptors, interstimulus intervals of 30 seconds were maintained. To minimize the effects of adaptation, after each episode, position of algometer was altered. Total area of stimulation was about 6x5 cm^2.

Results and discussion. At the first stage of the research, during the studies of sensitivity to the mechanical pain, it was identified that threshold of mechanical pressure is higher for primary, sensory-motor satiety than for physiological satiety. The difference between these two satiety levels was also demonstrated while testing mechanical pain threshold, where threshold for primary satiety equaled 350±2,6 kPa and for starvation level - 297±1,98 kPa. More significant results were present after analyzing individual resistance threshold to mechanical pain, particularly, a measured value during primary satiety made up 610±3,9 kPa and during physiological starvation 459±2,9kPa (Table).

According to this data, the cause of diminished pain sensitivity during primary satiety should be alterations of gastrointestinal tract that take place after food ingestion: Mechano- and chemoreceptors of initial segments in digestive system, particularly in stomach and in duodenum get irritated; that is followed by activation of several humoral factors and duodenal afferentation.

In 20-30 minutes after a mixed meal intake, pain sensitivity decreases; This is supposed to happen due to cholecystokinin release in duodenum. Cholecystokinin has some antinociceptive properties, it modulates neural system by endogenous opioid synthesis. This hypothesis is relevant to findings of the experiment on rats, where phenylquionone-induced seizures were extenuated by central and peripheral administration of cholecystokinin [29].

Besides the effects of cholecystokinin, satiety level influence on pain perception depends on cerebral level of serotonin [16]. Serotonin weakens nociceptive afferentation and is involved in morphine-like analgesia. It is proposed that impact of serotonin depends on the transport of amino acid tryptophan. Diets that are enriched with carbohydrates diminish pain sensitivity, induce calmness and sedation, the mechanism of which is thought to be insulin release – it stimulates taking up of neutral amino acids (including tryptophan). Even though this idea is expressed according to the study on animals [30], but the therapeutical role of tryptophan is also shown in humans. According to these studies, tryptophan rise in blood plasma is detected, but this change is not as significant to affect serotonin level in the brain.

The reliability of performed study is strengthened by evaluating mechanical pain by computerized algometer, where quantitative assessment of the parameters was automatically managed by pressing the remote control button. This type of assessing system is much more objective, than evaluating pain subjectively by verbal and qualitative tools. As for limitations of the study, individual characteristics of metabolism should be taken into consideration. In particular, food digestion and various substance release in response to meals (therefore, their effect on nociception) is not happening in exactly identical time periods for different individuals and therefore, correlation between pain perception and time after last food intake cannot be seen as a completely reliable factor.

For further research of satiety level influence on pain perception, it is recommended to analyze different types of experimental pain thresholds and resistance thresholds to pain. Measures of individual pain perception should be evaluated not only during starvation and primary satiety, but also during metabolic satiety as well. In addition, psychophysiological characteristics (different types of aggression) are desired to be taken into consideration as it will fill the gaps in our general knowledge about the phenomenon of pain and will also help us improve pain management resources. All of the above can be a subject for following studies and publications.

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SUMMARY

SENSITIVITY TO MECHANICAL PAIN BASED ON SATIETY LEVELS IN WOMEN

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The recent data have shown that in addition to biological and psychosocial mechanisms, individual pain perception can be altered by satiety level as well. Some studies confirm that there is a relationship between being fed by sucrose and hyperalgesia in animals, while others show that ketogenic diet can be associated with decreased pain sensitivity to thermal stimuli. It can be argued that the effect of satiety level on pain perception is mediated by gastrointestinal hormones and endogenous opioids. Pharmacological stimulation of kappa-opioid receptors decreases stress and promotes analgesia. Accordingly, these changes can be inhibited by applying antagonists.

Our study aims to assess pain perception induced by mechanical experimental irritation in women during different satiety levels in follicular phase of ovarian-menstrual cycle.

The sample of the study was comprised of volunteer students aged 18-23 (women, mean age 19,5±2,9).

Ovarian-menstrual cycle of the women participating in the research were evaluated using the questionnaires. All the studies were performed in the follicular phase of menstrual cycle (7-11 days of the cycle).

At the first stage, study was conducted in starvation state – after 10-12 hours after the last meal, the second stage – in primary, sensory-motor satiety 20-30 minutes after a mixed meal intake. Every participant has been offered a standard, mixed meal (including proteins, fats, carbohydrates).

Mechanical pain sensitivity was evaluated using computerized algometer - *AlgoMed (Medoc, Ltd, Ramat Yishai, Israel)*, which was delivering mechanical stimuli to the participants; Meanwhile, mechanical pressure threshold, mechanical pain threshold and pain tolerance threshold were determined.

According to this data, the reason of relatively diminished pain perception during primary satiety should be alterations of gastrointestinal tract that take place after food ingestion: Mechano- and chemoreceptors of initial segments in digestive system, particularly in stomach and in duodenum get irritated; that is followed by activation of several humoral factors and duodenal afferentation. In addition, by some authors duodenal release of cholecystokinin is believed to be hypothetical cause of decreased pain sensitivity after 20-30 minutes from the last mixed meal and is thought to have antinociceptive effect on endogenous opioid system.

Keywords: pain, mechanical pain, satiety levels, the follicular phase of menstrual cycle.

РЕЗЮМЕ

ПОКАЗАТЕЛИ МЕХАНИЧЕСКОЙ БОЛЕВОЙ ЧУВСТВИТЕЛЬНОСТИ В УСЛОВИЯХ РАЗЛИЧНОГО ПИЩЕВОГО СТАТУСА У ЖЕНЩИН

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Данные последних исследований показали, что, помимо биологических и психосоциальных механизмов, на степень индивидуального восприятия боли влияет также и пищевой статус организма. Исследования подтверждают существование взаимосвязи между кормлением животных сахарозой и гипералгезией, а в других показано, что кетогенная диета может быть связана со снижением болевой чувствительности к тепловым раздражителям. Утверждается, что влияние уровня насыщения на восприятие боли опосредуется желудочно-кишечными гормонами и эндогенными опиоидами. Фармакологическая стимуляция каппа-опиоидных рецепторов снижает стресс и способствует обезболиванию.

Целью исследования являлась оценка уровня восприятия боли, вызванной механическим экспериментальным раздражением у женщин в условиях различных пищевых статусов в фолликулярной фазе овариально-менструального цикла.

Субъектами исследования явились студентки-добровольцы в возрасте 18-23 лет (средний возраст 19,5±2,9 г.).

Фазы овариально-менструального цикла (ОМЦ) женщин, участвовавших в исследовании, определялись на основе анкетных данных. Все исследования проводились в фолликулярной фазе ОМЦ (7-11 день ОМЦ).

На первом этапе исследование проводилось в состоянии физиологического голода - спустя 10-12 часов после последнего приема пищи, натощак; на втором этапе - в состоянии первичного сенсомоторного насыщения т.е. спустя 20-30 минут после приема смешанной пищи. Каждому участнику предложено стандартное питание, включая белки, жиры, углеводы. Механическую болевую чувствительность оценивали с помощью компьютеризированного альгометра - AlgoMed (Medoc, Ltd, Израиль), который доставляет механические стимулы участникам; определялись пороги механического давления, механической боли и болевой толерантности.

Согласно полученным данным, механизм относительно слабого восприятия боли в условиях первичного насыщения должен основываться на изменениях в желудочно-кишечном тракте, происходящих после приема пищи: раздражение механо- и хеморецепторов начальных сегментов пищеварительной системы, особенно - желудка и двенадцатиперстной кишки, с последующей активацией определённых гуморальных факторов и дуоденальной афферентации. Некоторые авторы считают, что выброс холецистокинина в двенадцатиперстную кишку, гипотетически, может являться причиной снижения болевой чувствительности спустя 20–30 минут после приема пищи, оказывая антиноцицептивное действие через синтез эндогенных опиоидов.

რეზიუმე

მექანიკური ტკივილის მგრძნობელობის მაჩვენებლები სხვადასხვა კვებითი სტატუსის პირობებში ქალებში

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თბილისის სახელმწიფო სამედიცინო უნივერსიტეტი, ფიზიოლოგიის დეპარტამენტი

ბოლო წლებში ჩატარებული კვლევების საფუძველზე იკვეთება მოსაზრება,რომ,ბიოლოგიური და ფსიქოსოციალური მექანიზმების გარდა, ინდივიდის მიერ ტკივილის აღქმაზე შესაძლოა გავლენას ახდენდეს ორგანიზმის კვებითი სტატუსი. სხვადასხვა მონაცემების თანახმად, ცხოველებში საქაროზით კვება დაკავშირებულია ჰიპერალგეზიასთან, როგორც ფაზური, ასევე ტონური გამღიზიანებლის მიმართ; მეორე მხრივ, კეტოგენური კვება შესაძლოა ასოცირდებოდეს თერმული გამღიზიანებლით აღმოცენებული ტკივილის შემცირებასთან. სავარაუდოა, რომ კვებითი სტატუსის გავლენა ტკივილის მგრძნობელობაზე ემყარება საჭმლის მომნელებელი ჰორმონების და ენდოგენური ოპიოიდების მოქმედებას ორგანიზმში. კაპა-ოპიოიდური რეცეპტორების ფარმაკოლოგიური სტიმულაცია ამცირებს სტრესს, ხელს უწყობს ანალგეზიას.

წინამდებარე კვლევა მიზნად ისახავს მექანიკური ექსპერიმენტული გამღიზიანებლით განპირობებული ტკივილის შეგრძნების შეფასებას ქალებში ოვარიულ-მენსტრუალური ციკლის ფოლიკულურ ფაზაში სხვადასხვა კვებითი სტატუსის დროს.

კვლევის სამიზნე ჯგუფი წარმოდგენილი იყო 18-23 წლის ასაკის მოხალისე სტუდენტებით (ქალები, საშუალო ასაკი - 19,5±2,9 წელი).

კვლევაში ჩართული ქალების ოვარიულ-მენსტრუალური ციკლი (ომც) შეფასდა ანკეტირებით/გამოკითხვით. ყველა ქალის კვლევა ჩატარდა ომც-ს ფოლიკულურ ფაზაში (ომც-ს მე-7-11 დღე).

კვლევა პირველ ეტაპზე ჩატარდა უზმოდ,ფიზიოლოგიური შიმშილის მდგომარეობაში - საკვების მიღებიდან 10-12 საათის შემდეგ, მეორე კი - შერეული საკვების მიღებიდან 20-30 წუთის შემდეგ, ანუ პირველადი,სენსორულ-მოტორული მაძღრობის მდგომარეობაში. ყველა პრობანდისათვის შეთავაზებული იყო სტანდარტული, შერეული საკვები - ცილების, ცხიმების, ნახშირწყლების შემცველობით.

მექანიკური ტკივილის მგრძნობელობის შეფასება განხორციელდა პროგრამულად კონტროლირებადი ხელსაწყოთი AlgoMed (Medoc, Ltd, Israel), რომლის მეშვეობით სუბიექტებს მიეწოდებოდათ მექანიკური სტიმულები; პარალელურად აღირიცხებოდა მექანიკური მგრძნობელობის ზღურბლი, ტკივილის ზღურბლი და ტკივილისადმი მდგრადობის ზღურბლი (ტოლერანტობა).

კვლევის შედეგები იძლევა საფუძველს ჰიპოთეზისათვის, რომ პირველადი მაძღრობის მდგომარეობაში ტკივილისადმი მგრძნობელობის შედარებით დაქვეითებას საფუძვლად უდევს საჭმლის მომნელებელ ტრაქტში საკვების მოხვედრის შედეგად განვითარებული ცვლილებები: გასტროინტესტინური სისტემის საწყისი სეგმენტების, კუჭისა და თორმეტგოჯა ნაწლავის მექანო- და ქემორეცეპტორების

გაღიზიანება, რასაც მოსდევს სხვადასხვა ჰუმორული ფაქტორის აქტივაცია და დუოდენური აფერენტაცია. ამასთან, შერეული საკვების მიღებიდან 20-30 წთ-ში ტკივილისადმი მგრძნობელობის დაქვეითებაში ერთ-ერთ სავარაუდო მექანიზმად ავტორები განიხილავენ თორმეტგოჯა ნაწლავში ქოლეცისტოკინინის გამოყოფას, რასაც, სავარაუდოდ, ანტინოციცეპციური გავლენა აქვს ენდოგენური ოპიოიდების სინთეზის გამო.

FEATURES OF BONE METABOLISM AND THEIR INFLUENCE ON ARTERIAL WALL STIFFNESS IN POSTMENOPAUSAL WOMEN WITH CONTROLLED UNCOMPLICATED HYPERTENSION

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Cardiovascular disease occupies a leading place in the structure of morbidity and mortality [29]. With the global aging of the population, osteoporosis and cardiovascular diseases have become a major issue with considerable medical and socioeconomic burdens [13]. Age is an important determinant in the development of arterial hypertension (AH), which is largely associated with arterial consolidation due to the age-related changes and other risk factors [6]. Observational studies have reported an association between low serum vitamin D levels and elevated risk of cardiovascular disease (CVD), though such studies may not prove causation because of possible unmeasured confounding. Some findings concern the patients with osteoporosis who frequently suffer from vascular calcification, which was shown to predict both cardiovascular morbidity/mortality and osteoporotic fractures. Various common risk factors and mechanisms have been suggested to cause both bone loss and vascular calcification, including aging, estrogen deficiency, vitamin D and K abnormalities, chronic inflammation, oxidative stress, metabolic syndrome [24]. Major breakthroughs in molecular and cellular biology of bone metabolism and characterization of knockout animals with deletion of bone-related genes have led to the concept that common signaling pathways, transcription factors and extracellular matrix interactions may account for both skeletal and vascular abnormalities [12].

However, there seems to be a current lack of information on the nature of bone metabolism in patients with various diseases of the cardiovascular system, for example, arterial hypertension and arterial wall stiffness.

The aim of this study was to examine the features of bone metabolism and their influence on arterial wall stiffness in postmenopausal women with a controlled uncomplicated hypertension.

Material and methods. The study involved 44 women (main group) with the mean age of 69.04±0.72 years and a postmenopausal duration of 18.4±0.85 years, with uncomplicated arterial hypertension (AH) grade 2, and 30 healthy patients (control group), their mean age 69.3±1.21 years and postmenopausal duration of 19.4±1.18 years ($p>0.05$).

Inclusion criteria: females over 65 y.o. with a controlled AH of 1-2 grades, according to the office BP morning measurements. They took an antihypertensive therapy based on indapamide-retard + amlodipine at a dose of 1.5/5 mg /d or 1.5/10 mg/d with target blood pressure levels (<140/90 mm Hg).

Exclusion criteria: the presence of secondary hypertension; previous history of myocardial infarction and/or stroke; heart failure with NYHA above a functional class (FC) II signs of stable angina of the III-IV FC; left ventricular ejection fraction (LVEF)<50%; diabetes; congenital heart diseases; peripheral vascular disease; heart rhythm disturbances (permanent and persistent form of atrial fibrillation, frequent extrasystolic arrhythmia, ventricular paroxysms or ventricular tachycardia in the medical history, persistent sinus tachycardia); violation of atrioventricular conduction or sinus bradycardia (heart rate< 50 bpm) or weakness syndrome of the sinus node; impossibility to withdraw previous AHT; obesity with body mass index (BMI)>35 kg/m²; chronic kidney disease with GFR for EPI<60 ml/min/1.73 m2 and any other clinically relevant concomitant pathology; hyper- (> 5.5 mmol/L) and hypopotassemia (< 3.5 mmol/L).

Questionnaire-survey method was used to assess a nutritional status. Furthermore, patients were examined by a general clinical examination, routine laboratory clinical and biochemical studies, measurements of office bBP (brachial systolic, diastolic, pulse, mean BP (bSBP, bDBP, bPP, mean bBP) using a mechanical tonometer Microlife BP AG1-30. Applantation tonometry was performed using the SphygmoCor device AtCor Medical (Australia) and Doppler-Echo by the ultrasound diagnostic system of the Hitachi ALOKA Medical.

According to the pulse wave analysis by applanation tonometry [3], we determined central systolic, diastolic, pulse, and mean BP (respectively, cSBP, cDBP, cPP, mean cBP), augmentation pressure (AP), augmentation index (AIx), augmentation index, normalized for a pulse rate of 75 beats/min (AIx75), amplification pressure (PP ampl.), and measured carotid-radial (PVW rad.) and carotid-femoral pulse wave velocity (PWV fem.). The amplification pressure was calculated as the ratio between bPP and cPP (%) [19].

The FRAX-all and FRAX-hip technique was used to calculate the 10-year risk of hip fracture and major osteoporotic fractures (the Ukrainian version was developed under the guidance of Prof. Povoroznyuk V.V. at www.sheffield.ac.uk/FRAX/tool.aspx) [23].

Bone turnover markers in the peripheral blood (procollagen type 1 propeptide (P1NP), collagen type 1 cross-linked C-telopeptide (β-CTx)), parathyroid hormone (PTH) and vitamin D were defined by electrochemiluminescence method Eleksys 2010 analyzer (Roche Diagnostics, Germany), Cobas test systems. Levels of ionized calcium, phosphorus in serum (hexokinase method0 were assayed by the automatic biochemical analyzer Integra 400/800 ("Roche", Germany).

Vitamin D status was evaluated according to the latest classification [21,24], based on which vitamin D deficiency is diagnosed at 25 (OH) D in serum below 20 ng/ml, vitamin D deficiency at 25 (OH) D 20-30 ng/ml. A concentration of 25 (OH) D in the range of 30–50 ng/ml indicates an optimum level, and 50–100 ng/ml - a high level.

The bone mineral density (BMD) was examined using the "Hologic Discovery" apparatus. The following parameters of bone mineral density (BMD, g/cm^2) were determined: T score of the total body, lumbar spine L1-L4, femoral neck, radial bone. To assess the quality of bone tissue (Trabecular Bone Score - TBS), the TBS iNsight technique, developed by Med-Imaps (Bordeaux, France), was used.

Results and discussion. Within the framework of risk factor analysis for bone fractures, it was found that the calcium content in the actual diet (according to the questionnaires) in the main group was on average 245±21 mg/day, and in the control group - 268±23 mg/day. Thus, it was significantly reduced in both groups compared to the generally accepted norms.

At the time of the inclusion at this stage of the study, the target levels of blood pressure were reached, that is, the effect of elevated blood pressure on bone metabolism was excluded. Patients with hypertension and control group were compared by age, BMI, brachial and central blood pressure (Table 1).

bSBP, bDBP, bPP, mean bBP - brachial systolic, diastolic, pulse, mean blood pressure; cSBP, cDBP, cPP, mean cBP - central systolic, diastolic, pulse, and mean BP; AP-augmentation pressure; AIx-augmentation index; AIx75 - augmentation index, normalized for a pulse rate of 75 beats/min; PP ampl.- amplification pressure ; PWV rad., PWV fem. - carotid-radial and carotid-femoral pulse wave velocity.

At the time of inclusion, we revealed a significant increase in AP, AIx, AIx75 in the main group by 37.7%, 57.5%, 58.2% (Table 1, $p<0.001$) and a decrease in PPampl. by 20.8% (Table 1, $p<0.001$) compared to the control, which reflects the increase of the central PP due to the influence of the reflected wave, and characterizes the increased stiffness of arteries.

Patients of the main group, compared with the control group, at the time of inclusion in the study, had PWV rad. which was higher by 31% and PWV fem. by 32%, respectively (Table 1, all $p<0.001$). In hypertensives, PWV is an independent risk factor for cardiovascular death and all causes [30].

Table 1. Baseline data of BP and pulse wave indices in two groups of patients

Parameter	Main group n=44	Control group n=30
Age, years	69.04±0.72	69.3±1.21
Postmenopausal duration (PD), years	18.4±0.85	19.4±1.18
Duration of AH, years	17.0±0.86	–
BMI, kg/m²	28.9±0.55	27.6±1.11
bSBP, mm hg	123.6±1.95	121.2±1.85
bDBP, mmHg	78.3±1.28	79.3±1.51
bPP, mmHg	45.7±1.71	41.8±1.27
cSBP, mm Hg.	117.1±1.84	113.7±1.73
cDBP, mmHg	78.6±1.22	79.3±1.51
cPP, mmHg	38.4±1.53	35.1±1.15
HR (heart rates), bpm	66.0±1.09	72.7±1.34***
AP, mm Hg	14.5±0.87	9.03±0.59***
Alx, %	34.2±1.12	14.5±1.38***
Alx75, %	30.6±1.15	12.8±1.19***
PPampl, %	120.1±1.79	152.9±2.19***
PWV rad., m/s	10.0±0.28	6.89±0.26***
PWV fem., m/s	11.6±0.37	7.9±0.24***

*Statistically relevant difference in the scores between two groups * $p<0.05$; ** $p<0.01$; *** $p<0.001$*

Table 2. Baseline data of the examined groups

Parameter	Main group n=44	Control group n=30
TCh, mmol/l	6.28±0.18	4.6±0.1*
LDL cholesterol, mmol/l	3.83 ±0.17	1.73±0.14*
P1NP, ng/ml	55.12±4.45	58.32±3.24
Total vitamin D, ng/ml	23.21±1.1	29.18±2.12*
β – CTx, ng/ml	0.57±0.03	0.45±0.03*
PTH, ng/ml	67.9±3.75	39.56±1.14*
Ca++, mmol/l	1.27±0.02	1.3±0.02
Phosphorus, mmol/l	1.17±0.02	1.10±0.02

** - statistically relevant difference in the scores between two groups $p<0.05$*

The analysis of bone metabolism manifested the level of markers PINP, ionized calcium, phosphorus which did not differ in the comparison groups, whereas a significantly higher level of PTH was observed in patients of the main group, possibly as a result of vitamin D deficiency, more pronounced in patients with an AH.

Note the increase of the resorption marker activity in the main group, which was reflected by a likely increase in the marker β-CTx. This suggests the need for choosing osteoporosis treatment tactics.

The average level of vitamin D in patients with AH was lower by 20.7% compared to the healthy women. Vitamin D deficiency was found in 21 (47.7%) patients of the main group, deficiency - in 16 (36.4%), normal level - in 7 (15.9%), whereas in the control group, vitamin D deficiency was found in 6 (20.0%), insufficiency - in 4 (13.3%) patients. Secondary hyperparathyroidism was determined in the patients in the main group with vitamin D deficiency. Thus, patients with hypertension suffer a secondary hyperparathyroidism against the background of vitamin D deficiency, which may explain the additional negative effect on the alterations in blood vessel stiffness [5,18].

To evaluate the relationship between arterial stiffness, phosphorus-calcium metabolism, vitamin D, we conducted the Spearman correlation analysis. We found statistically significant correlations between the PWV fem. and the level of PTH, the PTH and the Alx, the PTH and the Alx 75%, the LDL level and Alx 75% (Table 3).

The main group results indicate the presence of hypercholesterolemia, a more pronounced deficiency of vitamin D, secondary hyperparathyroidism and accelerated bone tissue metabolism.

BMD disorders were found in 33 (75%) patients of the main group, 25 (56.8%) of them being women had osteopenia and 8 (18.2%) - osteoporosis. In the control group, BMD disorders

Table 3. Correlation between changes in BP and pulse wave values, arterial stiffness and bone metabolism in main group patients

Parameter	r
PPampl. – HR	0.44*
PTH – bPP	0.46*
PTH – PWV fem.	-0.44*
PTH - Alx	-0.31*
PTH – Alx 75%	-0.36*
P1NP- TCh	0.30**
P1NP- LDL	0.37**
Vitamin D - Age	-0.42*
Vitamin D - PD	-0.37*
Vitamin D – FRAX all	-0.4*
PPamp - LDL	0.36*
LDL – Alx 75%	-0.36*
LDL - AP	0.31*

** - the correlation is significant at the level of 0.05*

Table 4. Baseline data analysis of bone mineral density, FRAX of the examined groups

Parameter	Main group n=44	Control group n=30
FRAX all, %	5.94±0.44	4.44±0.12*
FRAX hip, %	1.72±0.27	1.13±0.09*
TBS, SD	1.27±0.02	1.30±0.03
BMD (L1-L4), g/sm^2	1.07±0.03	1.20±0.03*
T-score L1-L4, SD	-0.62±0.27	-0.20±0.24*
BMD femoral neck right, g/sm^2	0.84±0.02	0.97±0.03*
T-score femoral neck right, SD	-0.84±0.14	-0.23±0.17*
BMD femoral neck left, g/sm^2	0.84±0.02	1.02± 0.02*
T-score femoral neck left, SD	-0.8±0.15	-0.05± 0.2*
BMD Total body, g/sm^2	1.09±0.01	1.15±0.02*
T-score Total Body, SD	-0.5±0.16	-0.08±0.23
BMD Radius , g/sm^2	0.67±0.01	0.83±0.02*
T- score radius, SD	-1.04±0.18	-0.35±0.13*

** - statistically relevant difference in the scores between two groups p<0.05*

were found in 11 (36.7%) women: osteopenia in 7 (23.3%), osteoporosis in 4 (13.3%) (Table 3).

Average FRAX-all and FRAX-hip of the main group were significantly higher compared to the control group. This is explained by the presence of fractures in the anamnesis of 9 women (20.4%), included in the main group. Patients in the control group had no history of fractures.

Patients of the main group had a decrease in BMD at all the skeletal sites in (Table 4), compared with women without hypertension. The TBS bone quality index did not differ in the comparison groups.

To evaluate the relationship between arterial stiffness and BMD we conducted a Spearman correlation analysis (Table 5).

As revaled by the findings, the value of BMD total body, BMD radius, BMD femoral neck left, TBS, FRAX all were significantly decreased and associated with the increased parameters of applanation tonometry, in particular AP, AIx, AIx 75 (Table 5).

Correlation analysis in the control group did not reveal a significant correlation between the elastic-elastic properties of the arteries and the BMD indices.

Many epidemiological studies have shown that a low BMD and atherosclerosis appear to be related. However, their correlation is not completely clear after a full adjustment of shared confounders of atherosclerosis and bone metabolism [28].

Osteoporosis and vitamin D deficiency cause the impairments of bone density, strength and microarchitecture in older patients and increased risk of fragility fractures, and cause a significant morbidity and mortality [28].

Certain studies showed that vitamin D supplementation was not associated with reduced risks of MACE, myocardial infarction, stroke, cardiovascular mortality, or all cases of mortality. Additional trials of a higher-dose vitamin D supplementation, perhaps targeting members of older age groups and focusing on the CVD endpoints, such as heart failure, are of interest [4].

It was revealed in the studies [16] of older adults that vitamin D deficiency is associated with myocardial infarction and mortality. PTH excess is associated with heart failure. Vitamin D and PTH might influence cardiovascular risk through divergent pathways [10].

One of the studies [11] showed that in the older, predominantly postmenopausal South African women, BP, large artery stiffness and IMT were associated with calciotropic hormones and bone resorption, indicating a predisposition to arterial calcification. It is known that recombinant osteoprotegerin is a bioactive protein intended for use in the cell culture applications. Osteoprotegerin is an osteoblast-secreted decoy receptor that functions as a negative regulator of bone resorption. This protein specifically binds itself to its ligand, osteoprotegerin ligand, both of which are key extracellular regulators of osteoclast development. Studies of the mouse counterparts also suggest that this protein and its ligand play a role in lymph-node organogenesis and vascular calcification [26].

The study [17] confirmed an association between arterial stiffness and BMD in women. The other recent studies [20] showed a significant correlation between a vascular calcification and BMD.

There is a lack of data on the above-mentioned association in patients with CVD, namely with AH, for the possibility of substantiating common pathogenetic mechanisms between the development of osteoporosis and calcification of vessels, the role of secondary hyperparathyroidism.

Researchers have shown [7] that the arterial stiffness, as assessed by PWV, independently increased both with BP and with PTH, but BP remains the main driver of arterial stiffening.

Regarding the association between osteoporosis and atherosclerosis, the study [25] showed that the low BMD is associated with coronary atherosclerosis in the healthy postmenopausal women, independent of age and cardiovascular risk factors. Postmenopausal women with a decreased BMD may have a higher risk of developing coronary atherosclerosis.

The correlations we observed between PTH and PWVfem in women may indicate a possible relationship between media calcification (arteriosclerosis) and aortic atherosclerosis with the development of osteoporosis. This mechanism may be related to a violation of vitamin D. It is known that vitamin D deficiency is an important risk factor for the development of not only metabolic bone disease, but also of hypertension, obesity, diabetes, and its additional intake may significantly reduce the incidence of cardiovascular complications [5].

The key link in these processes is probably the disruption of the formation of the active metabolite of vitamin D, since the target organs for hypertension, diabetes are kidneys, and their lesion reduces the synthesis of 1α-hydroxylase - an enzyme through which 25-hydroxycholecalciferol (25 (OH) D3, calcidiol) in the kidneys is converted to the active form of vitamin D3-

Table 5. Correlation among pulse wave values, arterial stiffness and BMD in main group

Parameter	cPP, mmHg	AP, mmHg	Alx, %	Alx75, %	PPampl, %	PWV rad, m/s	PWV fem, m/s
FRAX all, %	**0.39***	**0.32***	**0.36***	0.37	**–0.44***	0.29	0.12
TBS, SD	–0.42	0.12	0.10	0.19	**0.32***	0.30	0.22
BMD (L1-L4), g/sm^2	0.26	0.01	–0.20	–0.18	0.19	0.14	**0.3***
T-score L1-L4, SD	–0.18	–0.18	**–0.57 ***	–0.13	0.28	0.02	0.24
BMD femoral neck right, g/sm^2	0.02	–0.02	0.11	0.30	0.21	0.14	0.22
T-score femoral neck right, SD	–0.18	–0.35	–0.27	–0.22	–0.09	0.15	–0.03
BMD femoral neck left, g/m^2	–0.04	–0.11	0.02	**0.36***	0.31	0.33	0.10
T-score femoral neck left, SD	–0.27	–0.16	–0.10	–0.27	0.01	0.03	–0.11
BMD Total body, g/sm^2	0.08	–0.09	**–0.41***	**–0.36***	0.12	0.24	0.14
T-score Total Body, SD	–0.26	–0.19	–0.10*	–0.12	0.18	–0.12	0.05
BMD Radius , g/sm^2	0.23	0.26	**-0.32**	**-0.32**	**-0.37***	0.15	0.12
T- score radius, SD	–0.29	–0.25	–0.30	–0.21	–0.04	–0.23	–0.13

** - the correlation is significant at the level of 0.05*

1,25 by dihydroxycholecalciferol (1,25 (OH) 2D3, calcitriol - D-hormone) [25]. Due to the hypovitaminosis of the D-hormone, hypocalcemia develops, which in turn leads to the development of secondary hyperparathyroidism, increasing the rate of bone tissue resorption, results in the development of OP and enhances calcium exit from the depot, increases its absorption in the intestine, and flow into the intestine. Alkaline phosphatase exchange is central in this process as a molecular marker of vascular calcification [22]. The production of endothelial cell vesicle matrix, which regulates mineralization in vascular intimacy and the media, stimulates smooth muscle cells (MMCs) [1]. Other cell types (eg. microvascular pericytes and adventitial fibroblasts) have the ability to generate a mineralized matrix and stimulate osteoblasts to differentiate, resulting in an increased calcification [22]. Arterial calcification may occur in the intimacy and media. Proinflammatory mediators cause an increase in LDL cholesterol concentration due to osteogenic differentiation of MMCs. Media calcification is associated with an advanced age, diabetes and chronic kidney disease, contributes to arterial stiffness, which increases the risk of adverse cardiovascular events [15]. In our study, we also obtained data on the deficiency of vitamin D, secondary hyperparathyroidism in patients with an uncomplicated hypertension, which may explain the mechanism of increase in the level of bone tissue resorption marker. Correlation between arterial stiffness (AP, AIx, AIx75) and BMD may indicate an association of media calcification, i.e. arteriosclerosis, and aortic atherosclerosis with development of OP [15]. In our opinion, the explanation for this phenomenon may be a disorder of vitamin D metabolism in the elderly women with hypertension, which we found in the study. It is known that vitamin D deficiency is an important risk factor for the development of not only metabolic bone disease, but also hypertension, obesity, diabetes, and its supplemental intake may significantly reduce the frequency of cardiovascular events [5]. We found statistically significant correlations between the PWVfem. and PTH levels. The value of BMD total body, BMD radius, BMD femoral neck left, TBS, FRAX-all were significantly decreased and associated with the increased parameters of applanation tonometry, in particular AP, AIx, AIx 75 . This probably indicates an association between vitamin D metabolism disorders due to the secondary hyperparathyroidism, progression of arterial rigidity and calcification of elastic fibers in women postmenopausal women with a controlled uncomplicated hypertension [2]. Correlations between total cholesterol (TCh), low density lipoprotein (LDL), and P1NP levels indicate the likelihood of hypercholesterolemia among bone turnover markers activity in the elderly patients with an uncomplicated hypertension.

Conclusions. The data obtained from the study of the parameters of applanation tonometry and the structural and functional state of bone tissue in patients with an uncomplicated hypertension, aged 69±3.30 years reveals the possibility of joint pathogenetic mechanisms of development the atherocalcinosis, increased vascular stiffness, developing osteoporosis. These processes were associated with the reduced level of 25(OH) D, hypercholesterolemia, secondary hyperparathyroidism, and determine the necessary selection of therapy for correcting the revealed disorders.

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SUMMARY

FEATURES OF BONE METABOLISM AND THEIR INFLUENCE ON ARTERIAL WALL STIFFNESS IN POSTMENOPAUSAL WOMEN WITH CONTROLLED UNCOMPLICATED HYPERTENSION

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The aim of this study was to investigate the features of bone metabolism and their influence on the arterial wall stiffness in postmenopausal women with a controlled uncomplicated arterial hypertension (AH).

The study involved 44 women (main group) with the mean age of 69.04±0.72 years and a postmenopausal duration of 18.4±0.85 years, suffering from an AH grade 2, and 30 healthy patients (control group), their mean age being 69.3±1.21 years, postmenopausal duration 19.4±1.18 years (p>0.05). All patients underwent general clinical and laboratory examination with determination of lipid level in blood. Pulse wave analysis (SphygmoCor) parameters , Bone mineral density (BMD) were assessed. The levels of 25(OH) D, parathyroid hormone, propeptide procollagen of type 1 aminoterminal (P1NP), b-isomerized C-terminal telopeptides (b-CTx), ionized calcium and phosphorus in serum were assessed.

The data obtained from the study of the parameters of applanation tonometry and the structural and functional state of bone tissue in patients with an uncomplicated hypertension at the age of 69±3.30 years manifest the likelihood of joint pathogenetic mechanisms of developing atherocalcinosis, increased vascular stiffness and impending osteoporosis.

Keywords: hypertension, arterial stiffness, osteoporosis.

РЕЗЮМЕ

ОСОБЕННОСТИ КОСТНОГО МЕТАБОЛИЗМА И ЕГО ВЛИЯНИЕ НА ЖЕСТКОСТЬ АРТЕРИАЛЬНОЙ СТЕНКИ У ЖЕНЩИН В ПОСТМЕНОПАУЗЕ С КОНТРОЛИРУЕМОЙ АРТЕРИАЛЬНОЙ ГИПЕРТЕНЗИЕЙ

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Цель исследования - изучить особенности метаболизма костной ткани и его влияние на жесткость артериальной стенки у женщин в постменопаузе с контролируемой неосложненной артериальной гипертензией.

В исследовании приняли участие 44 женщины (основная группа), средний возраст - 69,04±0,72 года, длительность постменопаузы 18,4±0,85 г., с артериальной гипертензией (АГ) 2 степени. Группу сравнения составили 30 здоровых женщин, средний возраст - 69,±1,21 г., длительность постменопаузы 19,4±1,18 г. (p>0,05). Исследуемым проведено общеклиническое и лабораторное обследование с определением уровня липидов в крови. Оценены параметры пульсовой волны (SphygmoCor), минеральная плотность костной ткани, уровни 25 (OH) D, паратиреоидного гормона, пропептида проколлагена аминотерминального типа 1, b-изомеризованных С-концевых телопептидов, ионизированного кальция и фосфора в сыворотке крови.

Данные, полученные при изучении параметров аппланационной тонометрии и структурно-функционального состояния костной ткани у пациентов с неосложненной АГ в возрасте 69±3,30 г., свидетельствуют о вероятности совместных патогенетических механизмах развития атерокальциноза, повышения жесткости сосудов и развития остеопороза.

რეზიუმე

ძვლოვანი მეტაბოლიზმის თავისებურებები და მოქმედება არტერიული კედლის სიმტკიცეზე მენოპაუზის ასაკის ქალებში კონტროლირებული არტერიული ჰიპერტენზიით

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კვლევის მიზანს წარმოადგენდა ძვლოვანი ქსოვილის თავისებურებების და მათი გავლენის შეფასება არტერიული კედლის სიმტკიცეზე მენოპაუზის ასაკის

ქალებში კონტროლირებული გაურთულებელი არტერიული ჰიპერტენზიით.

კვლევაში მონაწილეობა მიიღო 44 ქალმა (ძირითადი ჯგუფი) არტერიული ჰიპერტენზიის II ხარისხით, საშუალო ასაკი - 69,04±0,72 წ., მენოპაუზის ხანგრძლივობა - 18,4±0,85 წ. შედარების ჯგუფი შეადგინა 30 ჯანმრთელმა ქალმა, საშუალო ასაკი – 69±1,21 წ., მენოპაუზის ხანგრძლივობა - 19,4±1,18 წ. (p>0,05). პაციენტებს ჩაუტარდა ზოგადი კლინიკური და ლაბორატორიული კვლევა ლიპიდების დონის განსაზღვრით სისხლში. შეფასებულია პულსური ტალღის პარამეტრები (SphygmoCor), ძვლოვანი ქსოვილის მინერალური სიმკვრივე, 25 (OH) D-ის, პარა-თიროიდული ჰორმონის, ტიპი 1 ამინოტერმინალური პროკოლაგენის პროპეპტიდის, ხ-ი ზომერიზებული C–ტელოპეპტიდების, იონიზებული კალციუმის და ფოსფორის დონე სისხლის შრატში.

პარამეტრების შესწავლით მიღებული მონაცემები მიუთითებს ათეროკალცინოზის, არტერიული კედლის სიმტკიცის მომატების და ოსტეოპოროზის ერთობლივი პათოგენეზური მექანიზმების არსებობის შესაძლებლობის შესახებ.

CHARACTERISTICS OF DRUG RESISTANT TUBERCULOSIS IN GEORGIA (2015-2020)

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In 2019, an estimated 10 million people fell ill with tuberculosis (TB) worldwide (5.6 million men, 3.2 million women and 1.2 million children). A total of 1.4 million people died from TB in 2019 (including 208 000 people with HIV). Worldwide, TB is one of the top 10 causes of death and the leading cause from a single infectious agent (above HIV/AIDS). Multidrug-resistant TB (MDR-TB) remains a public health crisis and a health security threat. A global total of 206 030 people with multidrug- or rifampicin-resistant TB (MDR/RR-TB) were detected and notified in 2019, a 10% increase from 186 883 in 2018. Worldwide, only 57% of MDR-TB patients are currently successfully treated [1,2].

According to the World Health Organization (WHO), in 2018, the total number of notified TB Cases in Georgia was 2 590 (incidence – 65 cases per 100 000 population).

MDR-TB was diagnosed in 12% of new, and in 31% of previously treated cases. The treatment outcome was defined as successful in 65% of MDR/RR-TB and in 56% of XDR-TB cases started on second-line treatment in 2016 (cohorts – 339 and 55, respectively) [3].

For three decades, drug-resistant tuberculosis (TB) has posed grave challenges to patients, communities and global TB control efforts. Treatment of multidrug-resistant (MDR)-TB and extensively drug-resistant (XDR)-TB was relied on medications that are less potent and more toxic than first-line TB therapy, which is used for treatment of drug susceptible TB. Consequently, prolonged drug-resistant TB treatment was associated with frequent and severe side-effects. This led to the high rate of unfavorable treatment outcomes. Fortunately, in recent years TB world globally has several key innovations that, together, have brought us to a tipping point in revolutionizing the care of patients with MDR- and XDR-TB. In 2012, bedaquiline, the first new TB medication in more than 40 years, was approved by the US Food and Drug Administration (FDA). Approximately 6 months later, delamanid, in yet another new drug class, was approved by the European Medicines Agency [4]. Since 2019, based on WHO's recommendations toxic injectable agents (Kanamycin and Capreomycin) are removed from the DR-TB regimens and patients has the access to the shorter fully oral regimens [5-6]. From 2020, the new treatment regimen with next new drug - Pretomanid is recommended for treatment of XDR-TB patients [6].

Georgia as the part of TB world has always had access to the all previously and newly recommended treatment regimens and today, at the stage of transition from old to new DR-TB treatment, it's important to compare general characteristics of different DR-TB cohorts and to assess possibility to raise the effectiveness of DR-TB treatment in the future.

Material and methods. A retrospective cohort study was conducted with individual data of >18 years old DR-TB patients from 2015 -2020 cohorts, whose treatment outcome was defined until August 2020.

Considering the inclusion criteria, 1581 DR-TB patients (n=503[2015 cohort] + n=387[2016 cohort] + n=345[2017 cohort] + n=229[2018 cohort] + n=113[2019 cohort] + n=4[2020 cohort]), with known treatment outcomes were selected as study participants.

The study was conducted at the National Center for Tuberculosis and Lung Disease as a part of the Georgian National TB Programme. During the study period the treatment of DR-TB patients was provided based on latest WHO's recommendations and in different cohorts the different combinations of old, repurposed or new II line drugs with different duration was used.

Data variables were collected in relation to study objectives and included socio-demographic characteristics, laboratory data, data about susceptibility to the anti-TB drugs, treatment regimens and treatment outcomes.

Treatment was defined as successful in case of "Cured" and "Completed" treatment. "Failure", "Default", "Not Evaluated" and "Death" was defined as unsuccessful outcomes.

The data collected were analyzed by using of EasyStat (https://easystat.app). A descriptive analysis was performed for socio-demographic, behavioral and clinical characteristics. Bivariate and multivariate logistic regression analysis was used to measure the link between these characteristics and treatment outcomes. Odds ratios and their 95% confidence intervals were calculated. All the variables significant at $p<0.05$ in the bivariate analysis were included in the adjusted model.

Permission to carry out the study was obtained from the National Center for Tuberculosis and Lung Diseases (NCTLD) in Georgia. Local ethics approval was obtained from the Ethics Review Board of the NCTLD.

Results and discussion. The data of 2031 DR-TB patients from 2015 and 2020 cohorts were extracted from the National Tuberculosis Electronic Register. According to the inclusion criteria, 1581 DR-TB patients with known treatment outcomes were selected as the study participants (Fig. 1).

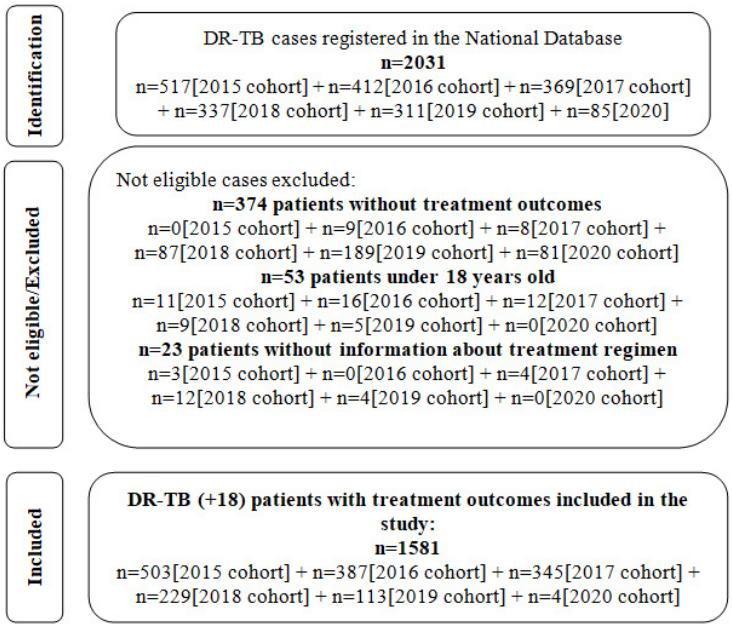


Fig. 1. Study flow chart

Table 1. Socio-demographic and clinical characteristics of the study participants, N=1581 (DR-TB patients with known treatment outcomes, Georgia, 2015–2020 cohorts)

Categories	Subcategories	Total N=1581
Gender (n,%)	Female	322 (20.4%)
	Male	1259 (79.6%)
Age (n,%)	>55	290 (18.3%)
	18-34	566 (35.8%)
	35-54	725 (45.9%)
Region (n,%)	High	675 (42.7%)
	Low	215 (13.6%)
	Middle	691 (43.7%)
Region_1 (n,%)	Adjara	130 (8.2%)
	Guria	22 (1.4%)
	Imereti	138 (8.7%)
	Kakheti	51 (3.2%)
	Kvemo Kartli	134 (8.5%)
	Mtskheta-mtianeti	23 (1.5%)
	Prison	89 (5.6%)
	Racha-Lechkhumi	3 (0.2%)
	Samegrelo	200 (12.7%)
	Samtskhe-Javakheti	45 (2.8%)
	Shida Kartli	71 (4.5%)
	Tbilisi	675 (42.7%)

Categories	Subcategories	Total N=1581
Employment (n,%)	Employed	186 (11.8%)
	Military	2 (0.1%)
	Unemployed	1393 (88.1%)
Alcohol abuse (n,%)	Excessive	127 (8%)
	Moderate	538 (34%)
	None	916 (57.9%)
Illicit Drug abuse (n,%)	No	1245 (78.7%)
	Unknown	256 (16.2%)
	Yes	80 (5.1%)
HIV (+) (n,%)	No	1479 (93.5%)
	Yes	102 (6.5%)
AFB (+) (n,%)	No	690 (43.6%)
	Not done	7 (0.4%)
	Yes	884 (55.9%)
Culture (+) (n,%)	No	26 (1.6%)
	Not Done	176 (11.1%)
	Yes	1379 (87.2%)
TB form (n,%)	EPTB	83 (5.2%)
	PTB	1498 (94.8%)
TB Case (n,%)	New Case	782 (49.5%)
	Previously Treated Case	799 (50.5%)
Hr (n,%)	_	305 (19.3%)
	No	58 (3.7%)
	Yes	1218 (77%)
RR (n,%)	_	96 (6.1%)
	No	109 (6.9%)
	Yes	1376 (87%)
Er (n,%)	_	344 (21.8%)
	No	279 (17.6%)
	Yes	958 (60.6%)
Zr (n,%)	_	797 (50.4%)
	No	245 (15.5%)
	Yes	539 (34.1%)
Sr (n,%)	_	398 (25.2%)
	No	80 (5.1%)
	Yes	1103 (69.8%)
Kmr (n,%)	_	312 (19.7%)
	No	778 (49.2%)
	Yes	491 (31.1%)
Cmr (n,%)	_	321 (20.3%)
	No	1076 (68.1%)

Categories	Subcategories	Total N=1581
	Yes	184 (11.6%)
Ofxr (n,%)	_	320 (20.2%)
	No	829 (52.4%)
	Yes	432 (27.3%)
Ptor (n,%)	_	1489 (94.2%)
	No	66 (4.2%)
	Yes	26 (1.6%)
Etor (n,%)	_	1536 (97.2%)
	No	38 (2.4%)
	Yes	7 (0.4%)
PASr (n,%)	_	497 (31.4%)
	No	985 (62.3%)
	Yes	99 (6.3%)
Csr (n,%)	_	1496 (94.6%)
	No	76 (4.8%)
	Yes	9 (0.6%)
Amx/clvr (n,%)	_	1574 (99.6%)
	No	7 (0.4%)
Cfzr (n,%)	_	1572 (99.4%)
	No	9 (0.6%)
Bdq and/or Dlm in the regimen (n,%)	Bdq	336 (21.3%)
	Bdq+Dlm	102 (6.5%)
	Dlm	140 (8.9%)
	No	1003 (63.4%)
New drug in the regimen	No	1003 (63.4%)
(Bdq or Dlm or Bdq+Dlm) (n,%)	Yes	578 (36.6%)
Fq in the regimen (n,%)	LFX	511 (32.3%)
	MFX	810 (51.2%)
	No	260 (16.4%)
Fq in the regimen (n,%)	No	260 (16.4%)
	Yes	1321 (83.6%)
Treatment outcome (n,%)	Successful	987 (62.4%)
	Unsuccessful	594 (37.6%)
Treatment outcome_1 (n,%)	Completed	141 (8.9%)
	Cured	846 (53.5%)
	Default	318 (20.1%)
	Died	88 (5.6%)
	Failure	133 (8.4%)
	Not Evaluated	55 (3.5%)

Legend:

HIV – human immunodeficiency virus	Sr- Streptomycin resistance	Csr- Ciclosiren resistance

PTB- Pulmonary Tuberculosis	Kmr – Kanamycin resistance	Amx/clvr – Amoxicillin/clavulanate resistance
EPTB – Extrapulmonary Tuberculosis	Cmr – Capreomycin resistance	Cfz- Clofazimine resistance
Hr – Isoniazid resistance	Ofxr – Ofloxacin resistance	Bdq - Bedaquiline
RR-Rifampicin resistance	Ptor – Prothionamide resistance	Dlm – Delamanid
Er – Ethambutol resistance	Etor- Ethionamide resistance	LFX – Levofloxacin
Zr- Pyrazinamide resistance	PASr- para-aminosalicylic acid resistance	MFX - Moxifloxacin

As the first stage the socio-demographic and clinical characteristics of selected 1581 (100%) DR-TB patients were summarized (Table 1). The majority of the DR-TB patients were Males (79.6%), from 35-54 age group (45.9%), from the regions with the middle (100-500 cases) TB prevalence (43.7%), unemployed persons (88.1%), without alcohol (57.9%) or illicit drug (78.7%) abuse.

Based on the study data 1498 (94.8%) patients were diagnosed as the pulmonary and 83 (5.2%) patients as the extrapulmonary TB cases. 782 (49.5%) patients were defined as the "New" and 799 (50.5%) as the "Previously treated" cases. HIV status of 102 (6.5%) DR-TB patients were positive.

Laboratory tests conducted at initial stage of diagnosis was AFB positive in 884 (55.9%) cases and Culture positive in 1379 (87.2%) cases. The data of the resistance to the key anti-TB drugs was following: Rifampicin resistance was detected in 1376 (87%), Isoniazid resistance in 1218 (77%) and Ofloxacin resistance in 432 (27.3%) cases.

Fluoroquinolones (Levofloxacin or Moxifloxacin) as the key anti-DR-TB drugs was used in the 1321 (83.6%) regimens. Bedaquiline (Bdq), or Delamanid (Dlm), or Bedaquiline and Delamanid together was used in the treatment regimen of 578 (36.6%) patients. Bdq as alone new drug was used in the majority of cases (336 (21.3%)), Dlm alone was used in 140 (8.9%) and Bdq and Dlm together in 102 (6.5%) cases.

Data of the new drugs (Bdq and/or Dlm) in the DR-TB regimens by cohort shows a picture of their implementation over the years (Table 2 and Fig. 2). If in 2015 the new drugs was prescribed in 18% cases, in 2019 this number was raised to the 84%. In 2016-2018, Bdq was prescribed in half of cases (52.7%-44.9%-46.6%) and in 2019 this number was raised to the 80%. Number of regimens with Dlm alone, or with Bdq and Dlm together was low comparing to the regimens with Bdq alone and this number in case of Dlm was decreased from maximum 36% (2017) to 3.2% (2019) and in case of Bdq+Dlm from maximum 29.6% (2018) to 16.8% (2019) of cases. These data is in line with WHOs recommendations based on which in period from 2015 to 2018 using of Bdq and Dlm separately or in combination was equally recommended. Since 2019, Bdq is recommended as the first choice "A" group drug, while Dlm, due the low safety, is recommended as the last choice "C" group drug [5-7].

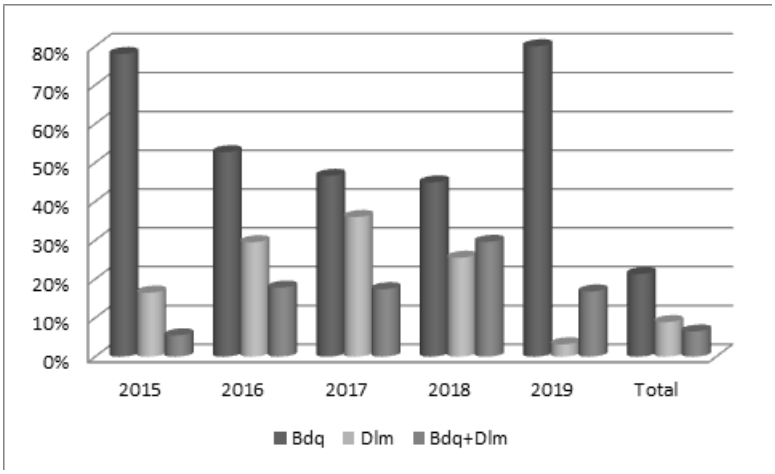
From the study population the successful treatment outcome was defined in 987 (62.4%) ("Cured" in 846 (53.5%) and "Completed" in 141 (8.9%) cases) and unsuccessful outcome in 594 (37.6%) cases ("Lost to follow-up" in 318 (20.1%), "Failure" in 133 (8.4%), "Death" in 88 (5.6%) and "Not evaluated" in 55 (3.5%) cases).

All key factors were analyzed for association with the treatment outcomes. The adjusted analysis was used for factors defined as significantly associated with the treatment outcomes (Table 3).

Table 2. Data of the new drugs in the DR-TB regimens and treatment outcomes by cohorts (2015-2020)

	New drug in the regimen			**Successful outcome**	**Unsuccessful outcome**
	Bdq	**Dlm**	**Bdq+Dlm**		
	n=91 (18%)				
2015 (n=503 100%))	71 (78%)	15 (16.5%)	5 (5.5%)	287 (57%)	216 (43%)
	n=129 (33.3%)				
2016 (n=387 (100%))	68 (52.7%)	38 (29.5%)	23 (17.8%)	260 (67%)	127 (33%)
	n=161 (47%)				
2017 (n=345 (100%))	75 (46.6%)	58 (36%)	28 (17.4%)	223 (64.6%)	122 (35.4%)
	n=98 (43%)				
2018 (n=229 (100%))	44 (44.9%)	25 (25.5%)	29 (29.6%)	150 (65.5%)	79 (34.5%)
	n=95 (84%)				
2019 (n=113 100%))	76 (80%)	3 (3.2%)	16 (16.8%)	67 (59.3%)	46 (40.7%)
	n=4 (100%)				
2020 (n=4 (100%))*	2 (50%)	1 (25%)	1 (25%)	0 (0%)	4 (100%)
	n=578 (36.6%)				
Total (n=1581 (100%))	336 (21.3%)	140 (8.9%)	102 (6.5%)	987 (62.4%)	594 (37.6%)

**Study population was selected from the patients with known treatment outcomes. By August 2020, in the National Tuberculosis Electronic Register the treatment outcomes of 4 patients were entered only*



Legend: Bdq- Bedaquiline; Dlm - Delamanid
Fig. 2. New drugs in the DR-TB regimens by years (2015-2019)

Table 3. Factors associated with TB treatment outcomes (DR-TB cases, Georgia, 2015–2020 cohorts)

Categories	Subcategories	Total N=8468	Successful N=6833	Unsuccessful N=1635	Bivariate			Multivariate		
					OR	95% CI	p	OR	95% CI	p
Gender (n,%)	Female	322 (20.4%)	247 (25%)	75 (12.6%)	2.31	[1.74, 3.06]	<0.001	1.78	[1.33, 2.39]	<0.001
	Male	1259 (79.6%)	740 (75%)	519 (87.4%)	1			ref.	ref.	ref.
Age (n,%)	>55	290 (18.3%)	179 (18.1%)	111 (18.7%)	1	-	-			
	18-34	566 (35.8%)	357 (36.2%)	209 (35.2%)	1.06	[0.79, 1.42]	0.699			
	35-54	725 (45.9%)	451 (45.7%)	274 (46.1%)	1.02	[0.77, 1.35]	0.886			
Region (n,%)	High	675 (42.7%)	414 (41.9%)	261 (43.9%)	1	-	-			
	Low	215 (13.6%)	130 (13.2%)	85 (14.3%)	0.96	[0.7, 1.32]	0.82			
	Middle	691 (43.7%)	443 (44.9%)	248 (41.8%)	1.13	[0.9, 1.4]	0.289			
Employment (n,%)	Employed	186 (11.8%)	143 (14.5%)	43 (7.2%)	1	-	-			
	Military	2 (0.1%)	2 (0.2%)	0 (0%)	Inf	[0.06, Inf]	1			
	Unemployed	1393 (88.1%)	842 (85.3%)	551 (92.8%)	0.46	[0.32, 0.66]	<0.001			
Alcohol abuse (n,%)	Excessive	127 (8%)	71 (7.2%)	56 (9.4%)	1	-	-			
	Moderate	538 (34%)	314 (31.8%)	224 (37.7%)	1.11	[0.75, 1.63]	0.614			
	None	916 (57.9%)	602 (61%)	314 (52.9%)	1.51	[1.04, 2.2]	0.0303			
Illicit Drug abuse (n,%)	No	1245 (78.7%)	819 (83%)	426 (71.7%)	1	-	-			
	Unknown	256 (16.2%)	131 (13.3%)	125 (21%)	0.55	[0.42, 0.72]	<0.001			
	Yes	80 (5.1%)	37 (3.7%)	43 (7.2%)	0.45	[0.28, 0.71]	<0.001			
HIV (+) (n,%)	No	1479 (93.5%)	946 (95.8%)	533 (89.7%)	2.64	[1.75, 3.98]	<0.001	2.33	[1.53, 3.55]	<0.001
	Yes	102 (6.5%)	41 (4.2%)	61 (10.3%)	1			ref.	ref.	ref.
AFB (+) (n,%)	No	690 (43.6%)	435 (44.1%)	255 (42.9%)	1	-	-			
	Not done	7 (0.4%)	4 (0.4%)	3 (0.5%)	0.78	[0.13, 5.38]	0.714			
	Yes	884 (55.9%)	548 (55.5%)	336 (56.6%)	0.96	[0.78, 1.17]	0.669			
Culture (+) (n,%)	No	26 (1.6%)	16 (1.6%)	10 (1.7%)	1	-	-			
	Not Done	176 (11.1%)	106 (10.7%)	70 (11.8%)	0.95	[0.41, 2.2]	0.898			
	Yes	1379 (87.2%)	865 (87.6%)	514 (86.5%)	1.05	[0.47, 2.34]	0.901			
TB Form (n,%)	EPTB	83 (5.2%)	49 (5%)	34 (5.7%)	0.86	[0.55, 1.35]	0.512			
	PTB	1498 (94.8%)	938 (95%)	560 (94.3%)	1					

Categories	Subcategories	Total N=8468	Successful N=6833	Unsuccessful N=1635	Bivariate			Multivariate		
					OR	95% CI	p	OR	95% CI	p
TB Case (n,%)	New Case	782 (49.5%)	575 (58.3%)	207 (34.8%)	2.61	[2.11, 3.22]	<0.001	2.34	[1.88, 2.91]	<0.001
	Previously Treated Case	799 (50.5%)	412 (41.7%)	387 (65.2%)	1			ref.	ref.	ref.
Hr (n,%)	_	305 (19.3%)	197 (20%)	108 (18.2%)	1	-	-			
	No	58 (3.7%)	38 (3.9%)	20 (3.4%)	1.04	[0.58, 1.88]	0.892			
	Yes	1218 (77%)	752 (76.2%)	466 (78.5%)	0.88	[0.68, 1.15]	0.358			
RR (n,%)	_	96 (6.1%)	59 (6%)	37 (6.2%)	1	-	-			
	No	109 (6.9%)	77 (7.8%)	32 (5.4%)	1.51	[0.84, 2.7]	0.165			
	Yes	1376 (87%)	851 (86.2%)	525 (88.4%)	1.02	[0.66, 1.56]	0.94			
Ofxr (n,%)	_	320 (20.2%)	209 (21.2%)	111 (18.7%)	1	-	-			
	No	829 (52.4%)	544 (55.1%)	285 (48%)	1.01	[0.77, 1.33]	0.921			
	Yes	432 (27.3%)	234 (23.7%)	198 (33.3%)	0.63	[0.47, 0.85]	0.00213			
New drug in the regimen (n,%)	No	1003 (63.4%)	595 (60.3%)	408 (68.7%)	0.69	[0.56, 0.86]	<0.001			
	Yes	578 (36.6%)	392 (39.7%)	186 (31.3%)	1					
Fq in the regimen (n,%)	No	260 (16.4%)	147 (14.9%)	113 (19%)	0.74	[0.57, 0.98]	0.0319			
	Yes	1321 (83.6%)	840 (85.1%)	481 (81%)	1					

Legend: ref. – reference category

In bivariate analysis, TB treatment success was positively associated with the female gender (OR 2.31; 95% CI [1.74–3.06]; p<0.001); new case (OR 2.61; 95% CI [2.11-3.22]; p<0.001); and with HIV negative status (OR 2.64; 95% CI [1.75–3.98]; p<0.001).

Adjusted analysis confirms significant association of a successful TB treatment outcome with the female gender (adjusted OR 1.78, 95% CI: 1.33 – 2.39, p<0.001), new TB case (adjusted OR 2.34, 95% CI: 1.88–2.91, p<0.001) and with HIV negative status (OR 2.33; 95% CI 1.53–3.55; p<0.001). Association of a treatment outcome with other key factors, including "New drugs in the regimen" was not found.

Data of the new drugs (Bdq and/or Dlm) in the DR-TB regimens in total and by cohort was assessed to evaluate association between regimens with the new drugs and successful treatment outcomes. Based on bivariate and multivariate analysis association between these factors ("New drugs in the regimen" and "Treatment outcomes") was not found. This may be explained by the fact that until 2019, Bdq and/or Dlm mostly were prescribed in combination with less effective and safe drugs, which based on the latest WHO's recommendations are defined as the last choice "C" group drugs. Since 2019, Bdq is mostly prescribed in combination with other "A" and "B" group drugs, but the number of DR-TB patients on such regimens with known treatment outcomes was low in the study population and does not allow reliable assessment. Further studies are necessary to assess complete data of the patients on new DR-TB regimens and its association with the treatment outcomes.

Statistical analysis also excluded association between treatment outcome and factors such as Alcohol or Illicit drug abuse and etc. This suggests that the factors that are proven to be associated with the increased risks for development of TB disease, does not have the statistically significant impact on the overall rate of the unfavorable treatment outcomes. But to what extent the other factors, such as effectiveness of the DR-TB regimens may affect on the treatment outcome, should be evaluated through the complete assessment and comparison of past, current and potentially applicable DR-TB regimens in the future.

Study data show, that despite of many efforts the rate of "Lost to follow-up" is still high in DR-TB cases (318 (20.1%)) and filling this gap still requires additional activities.

Study also shows that rate of "New" and "Previously treated" DR-TB cases are almost equal (782 (49.5%) "New" and 799 (50.5%) "Previously treated" cases). This indicates the high risk of DR-TB transmission and necessity to improve the quality of infection control measures at country level.

Adjusted analysis show, that "Female gender", "New case" and HIV negative status are significantly associated with successful TB treatment outcomes, which is in line with the results of previously conducted similar studies [8,9].

Limitations of the study. As mentioned, the study population was selected from the patients whose treatment outcomes were known by August 2020. 189 patients from 2019 cohort and 81 patients from 2020 cohorts with unknown treatment outcomes were excluded from the study (the treatment outcomes of only 4 patients from 2020 cohort were entered in the National Tuberculosis Electronic Register). As a result the study contains limited information about DR-TB patients from the last two cohorts, where the new DR-TB regimens most widely were used. This is the main reason why the new DR-TB regimens and its association with treatment outcomes were not fully assessed and this is the main limitation of the study.

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SUMMARY

CHARACTERISTICS OF DRUG RESISTANT TUBERCULOSIS IN GEORGIA (2015-2020)

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The aim of the study was to assess general characteristics of drug resistant tuberculosis and its association with treatment outcomes in Georgia. A retrospective cohort study was conducted among 1581 DR-TB adult (18+) patients, from 2015 - 2020 cohorts, whose anti-tuberculosis treatment outcomes was known. Adjusted analysis of the study participants data [1581 (100%)] shows significant association of a successful TB treatment outcome with the "Female gender" (adjusted OR 1.78, 95% CI: 1.33 – 2.39, p<0.001), "New TB case" (adjusted OR 2.34, 95% CI: 1.88–2.91, p<0.001) and with "HIV negative status" (OR 2.33; 95% CI 1.53–3.55; p<0.001).

Based on bivariate and multivariate analysis of the study data, the significant association of a treatment outcome with other key factors, including "New drugs in the regimen" was not found.

Since the programmatic using of the new effective DR-TB regimens are widely recommended only from 2019, the treatment outcomes of all patients on these regimens are still not defined. Further studies are necessary to assess complete data of the patients on new DR-TB regimens and its association with the treatment outcomes.

Keywords: Tuberculosis, Drug Resistant Tuberculosis (DR-TB), New anti-DR-TB drugs/regimens, TB treatment outcomes.

РЕЗЮМЕ

ХАРАКТЕРИСТИКА ЛЕКАРСТВЕННО-УСТОЙЧИВОГО ТУБЕРКУЛЕЗА В ГРУЗИИ (2015-2020)

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Целью исследования явилась оценка общих характеристик лекарственно-устойчивого туберкулеза и результатов его лечения в Грузии.

Проведено ретроспективное (2015-2020 гг.) когортное исследование 1581 пациента старше 18 лет с лекарственно-устойчивым туберкулезом (ЛУ-ТБ), чьи исходы противотуберкулезного лечения были известны. Уточненный анализ данных участников исследования показал статистический достоверную связь успешного исхода лечения туберкулеза у женщин (уточненное ОШ 1.78, 95% ДИ: 1.33 – 2.39, p<0,001), с "новым случаем туберкулеза" (уточненное ОШ 2.34, 95% ДИ: 1.88-2.91, p<0,001) и с "ВИЧ отрицательным статусом" (уточненное ОШ 2.33, 95% ДИ: 1.53-3.55, p<0,001). Двухмерный и многомерный анализы результатов исследования достоверной связи исхода лечения с другими ключевыми факторами, включая «новые препараты в схеме», не выявили. Поскольку программное использование новых эффективных схем лечения ЛУ-ТБ широко рекомендуется только с 2019 года, результаты лечения по этим схемам по сей день не определены. Авторы считают целесообразным проведение дальнейших исследований с целью оценки эффективности новых схем лечения ЛУ-ТБ.

რეზიუმე

რეზისტენტული ტუბერკულოზის მახასიათებლები საქართველოში (2015-2020)

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კვლევის მიზანს წარმოადგენს რეზისტენტული ტუბერკულოზის ძირითადი მახასიათებლების და მათი

მკურნალობის გამოსავალთან ასოცირების შეფასება.

2015-2020 წწ. ჩატარდა რეტროსპექტული კოჰორტული კვლევა 1581 მოზრდილი (18+) პაციენტთის, რომელთა მკურნალობის გამოსავალი ცნობილი იყო.

კვლევის მონაწილეთა მონაცემების დაზუსტებულმა ანალიზმა აჩვენა, რომ რეზისტენტული ტუბერკულოზის საწინააღმდეგო მკურნალობის წარმატებული გამოსავალი სარწმუნოდ ასოცირდება „მდედრობით სქესთან“ (adjusted OR 1.78, 95% CI: 1.33 – 2.39, p<0.001), „ახალ შემთხვევასთან“ (adjusted OR 2.34, 95% CI: 1.88–2.91, p<0.001) და „ახალ აივ ნეგატიურ სტატუსთან” (OR 2.33; 95% CI 1.53–3.55; p<0.001).

კვლევის მონაცემთა ბი- და მულტივარიაციულ ანალიზზე დაყრდნობით, მკურნალობის გამოსავლის სარწმუნო ასოცირება რეზისტენტული ტუბერკულოზის სხვა საკვანძო მახასიათებლებთან, მათ შორის „ახალი ტუბსაწინააღმდეგო მედიკამანტების შემცველი რეჟიმებით მკურნალობასთან“, არ გამოვლინდა.

რეზისტენტული ტუბერკულოზის ახალი, ეფექტური რეჟიმებით პროგრამული მკურნალობა ფართოდ რეკომენდებულია 2019 წლიდან, აქედან გამომდინარე, სადღეისოდ ამ რეჟიმებზე მყოფი ყველა პაციენტის მკურნალობის გამოსავალი არ არის შეფასებული. ავტორებს მიზანშეწონილად მიაჩნია რეზისტენტული ტუბერკულოზის სამკურნალო ახალ რეჟიმებზე მყოფი პაციენტების სრული მონაცემების და მათი მკურნალობის გამოსავალთან ასოცირების სრულყოფილად შესასწავლად დამატებითი კვლევების ჩატარება.

CYPRESS POLLEN SESITIZATION IN GEORGIA: CLINICAL AND MOLECULAR CHARACTERISTICS

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Cypress pollen allergy is a widely distributed, highly prevalent and severe winter pollinosis [3] that may be caused by several Cupresaceae species around the Mediterranean basin, in North America and Asia. Exposure to cypress pollen has increased steadily over the last few decades and the prevalence of allergy to cypress pollen has also dramatically increased from 0.6% to 9.8% in the general population and from 9% to 35% in allergic patients, probably because of the allergen load has become more intense [2]. The first cases of cypress pollinosis were described in South Africa in 1945 and in France in 1962. During the following decades, cypress species have been extensively planted for ornamental purpose, since they have low water needs, fast growth and have a low-cost maintenance [3]. These plants are anemophilous, shedding large amounts of pollen, being an important cause of allergic diseases, especially during the winter [5]. This increased exposure has been responsible for the increase in prevalence of sensitization and clinical manifestations of cypress pollen allergy [7].

Concerning the clinical expression, the main clinical symptom associated with allergy to cypress tree pollen is rhinitis, often associated with disabling conjunctivitis, whereas the incidence of asthma is generally lower than in patients sensitized to other allergenic sources [6]. The allergic reactions to *Cupressaceae* pollen, which usually occur in winter, could have overlapping symptoms with common cold or influenza [5]. Cypress pollinosis symptoms are often hard to control. Caimmi reported a 57.9% of cypress pollen allergic patients needing immunotherapy to control their symptoms [2].

In Mediterranean areas, C. sempervirens (Italian cypress or Mediterranean cypress) is by far the most common pollinating species. It accounts for half of the total pollination level [3]. According to Georgian pollen count data, cypress pollen is the major aeroallergen component in winter and early spring [1], but there have been no studies regarding the influence of cypress pollen high exposure in patients with pollen allergy. Thus, the objective of the study was to evaluate cypress pollen allergy in Georgia and describe clinical characteristics and the molecular profile of sensitization.

Material and methods. Patients attended to allergy clinic with suspected cypress pollen allergy (n=492) were included. Diagnostic workup was performed according to local guidelines, specific IgE antibody against cypress allergen was performed using ImmunoCAP and ISAC assay platform. Primary cypress pollen reactivity was confirmed by measuring IgE specific to *Cupressus arizonica* component Cup a 1 (t226) and *Cupressus Sempervirens* extract (t23) by ImmunoCAP (ThermoFisher, Uppsala, Sweden). IgE levels exceeding 0.35 kU/L were considered positive. The allergen microarray assay (ImmunoCAP ISAC; ThermoFisher) was used to analyse the specific IgE repertoire of cypress positive patient's serum. ISAC is a test for semi-quantitative determination of IgE in serum samples. The solid phase in this test is provided by the surface of a plate on which 112 components (43 native and 69 recombinant) have been adsorbed and arranged in triplets. Antibody levels were expressed in standardised units, ISU-E (ISAC Standardised Unit for specific IgE). The measured values ranged from 0.3 to 100 ISU-E, and values ≥ 0.30 ISU-E were considered to be positive results.

Symptoms Diary. Cypress positive patients were interviewed with the seasonal symptom's questionnaire. The severity of eye (itching and/or tear flow and/or conjunctival redness), nose (sneezing and/or runny nose and/or blocked nose), and bronchial (cough and/or wheezing and/or asthma) symptoms were recorded on a 4-point scale (0, no symptoms; 1, mild symptoms; 2, moderate symptoms; 3, severe symptoms). They were asked regarding medication use (antihistamines, local treatment for conjunctivitis and/or rhinitis and asthma) during the cypress season.

Plant Aeroallergens/Pollen Monitoring. The airborne pollen

monitoring was performed with a Burkard Seven Day Volumetric Spore-trap (Burkard Manufacturing Co Ltd, UK) during the seasons of 2019-2020, following the recommendations of European Aerobiology Society [4]. This trap is placed on the roof of Botanical Institute building of the Ilia State University of Georgia, Tbilisi, approximately 15 m above ground level. A strip of silicone-coated Melinex tape was exposed to the air for trapping the plant aeroallergens, and was changed once a week. The exposed tape was cut into 48 mm segments representing 24 h periods. These segments were mounted on microscopic slides using Mowiol mixed with a stain (basic fuchsine) to enable visualization under a high resolution light microscope at 400× magnification. Pollens concentration was calculated and expressed as the number of pollen grains per cubic meter of air (p/m^3). The criteria used to describe the dinamics and patterns of airborne pollen are as follows: the peak pollen concentration; pollen index, which was defined as the total number of pollen grains during the pollination period; the corresponding to the beginning and end of pollination (season begins at 1%, ends at 95% of total sum); the duration of the pollen season [3].

We summarized data as numbers (n) and frequencies (%) if they were categorical and as mean or median and standard deviation or interquartile range if quantitative. Data were entered and analyzed using the Statistical Package for Social Sciences database (SPSS, Inc., Chicago, IL, USA).

Results and discussion. A positive cypress diagnostic test was detected in 183 individuals from 492 allergic patients included in the study (37.2% of studied cases). Demographic and clinical characteristics of studied cypress positive patients are summarized in Table 1. Among cypress positive cases the male/female proportion was 108/75 (59%/41%), mean age (with standard deviation) – 22.7±1.4 years (for children (<18 years old) - 8.8±3.7 and adults - 33.2±9.8). The mean sIgE was 4.02±0.48 kUA/l for C. Sempervirens extract and 29.2±3.23 kUA/l for cup a 1. Diagnosis of allergic rhinitis has occurred in 92.3%, atopic conjunctivitis – 56.8%, asthma – 6% and atopic dermatitis – 10.4%.

Table 1. Baseline characteristics of study patients

Demographic characteristics	
age (±Std. Deviation)	22.7±1.4
Children (<18 years old)	8.8±3.7
Adults (≥18 years old)	33.2±9.8
Female	41%
Male	59%
IgE results	
ImmunoCAP Cypress Sempervirens t23 (KUA/l±S.D.mean)	4.02±0.48
ImmunoCAP nCup a 1 Cypress arizonica t226 (KUA/l±S.D.mean)	29.2±3.23
Diagnosis	
Acute atopic conjunctivitis	56.8%
Allergic rhinitis	92.3%
Asthma	6.0%
Atopic dermatitis	10.4%
Urticaria	7.1%
Cough	8.2%
Angioneurotic edema	6.0%

Table 2. Frequency of reported symptoms during the allergy season

List of symptoms	**Symptoms (n/%)**	**Severe symptoms (n/%)**
ocular itching	145/82,9%	82/44.8%
sneezing	145/82,9%	106/57.9%
nasal obstruction	142/81,1%	101/55.2%
rhinorrhea	136/77,7%	90/49.2%
ocular redness	116/66,3%	62/33.9%
watery eyes	99/56,6%	39/21.3%
nasal itching	82/46,9%	43/23.5%
shortness of breath	39/22,3%	8/4.4%
dry cough	39/22,3%	13/7.1%
foreign body sensation (eye)	33/18,9%	11/6.0%
chest tightness	16/9,1%	5/2.7%
wheezing	15/8,6%	7/3.8%

Table 3. Cypress pollen count data (average values for 2019-2020 year)

Season Start (date)	Peak day (date)	Season End (date)	Peak value (p/m$^{3)}$	Pollen index	Duration (#days)	% total annual amount of pollens
February 7	March 12	April 6	2811	19823	145	48,7%

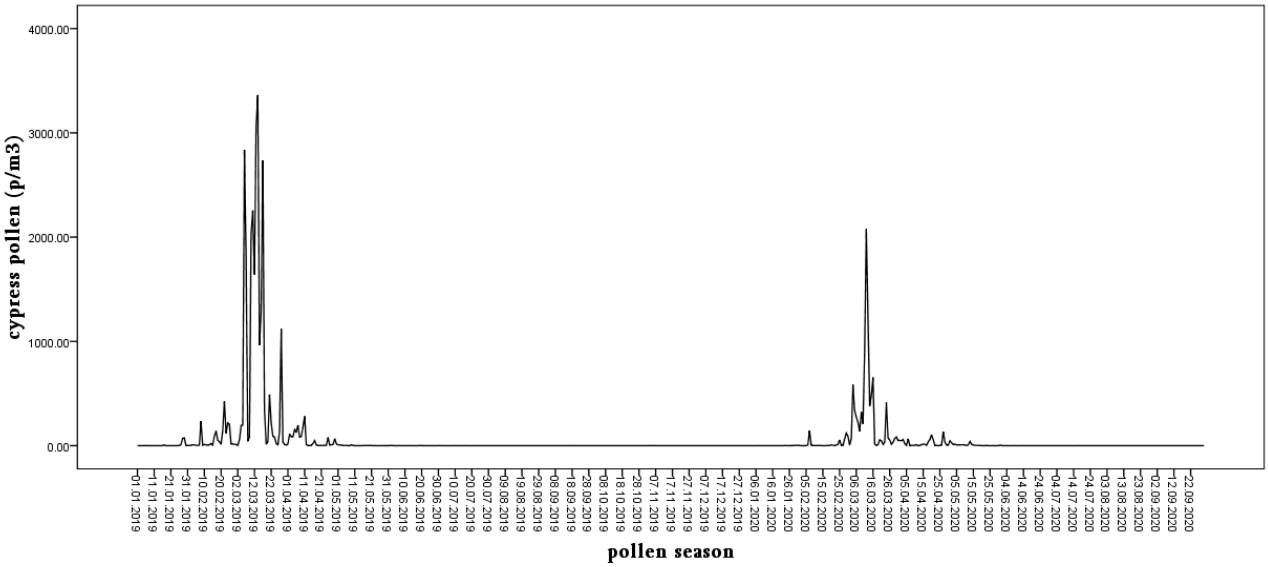

Fig. 1. The major distribution of cypress pollen during its pollination at 2019-2020

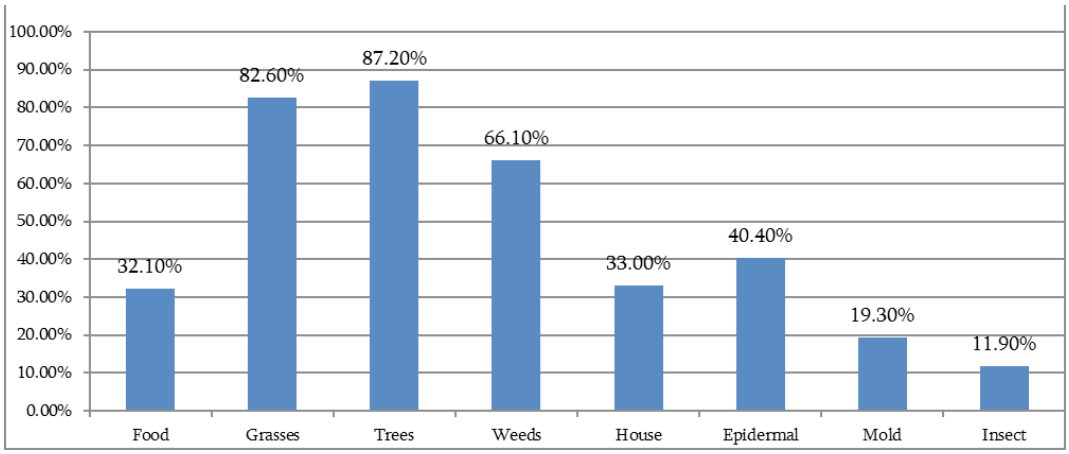

Fig. 2. Sensitization to mainly species-specific components`

From cypress positive 183 patients only 17 patients (9.2%) had no symptoms during the cypress season. As reported in Table 2, the most frequent symptoms were sneezing and ocular itching (82.9% of patients for both symptoms), rhinorrhea was reported in 77.7% of cases and dry cough and shortness of breath - only in 22.3% (each symptoms). More than half of frequent symptoms were reported as severe. Concerning the medication use during the pollen season, 65% of patients used eye drops, 86% - nose drops, 89.6% - antihistamine medications and only 6% the inhalation steroids.

Environmental Data. Table 3 describes the cypress pollen count data in Tbilisi during last two years and the changes in daily pollen levels are shown in Fig. 1. The main cypress pollen season in Tbilisi occurs from beginning of February to beginning of April, being March the month with the highest recorded airborne pollen concentration (average 2811 pollen grains p/m$^{3/}$ day). The average total pollen index for cypress was the total 19823 p/m^3. *Cupressus* pollen accounts for 48.7% of total annual pollen in Tbilisi, season duration is 145 days. The graph shows that the majority of Cupressaceae pollen is produced between January and April.

Sensitization profile. According to ISAC results all patients were poly-sensitized. The molecular profiles of *Cupressaceae* pollen-sensitized patients revealed followings (Fig. 2.): 1) sensitization to mainly species-specific food components (including storage proteins) – 32.1%, 2) sensitization to mainly species-specific aeroallergen components among them grass pollen – 82.6%, tree pollen components – 87.2%, weed pollen – 66.1%, mites – 33.0%, epidermal – 40.4%, mold – 19.3%; 3) sensitization to other mainly species-specific component (insect venom) – 11.9%.

Fig. 3 shows the sensitization to cross-reactive components: low co-sensitization to serum albumin (5,50%), tropomyosin (1,80%), thaumatin like protein (11%) and polcalcin (3.7%); moderate co-sensitization to LTP (20.2%), PR-10 (29.4%), Profilin (24.8%) and CCD (21.1%).

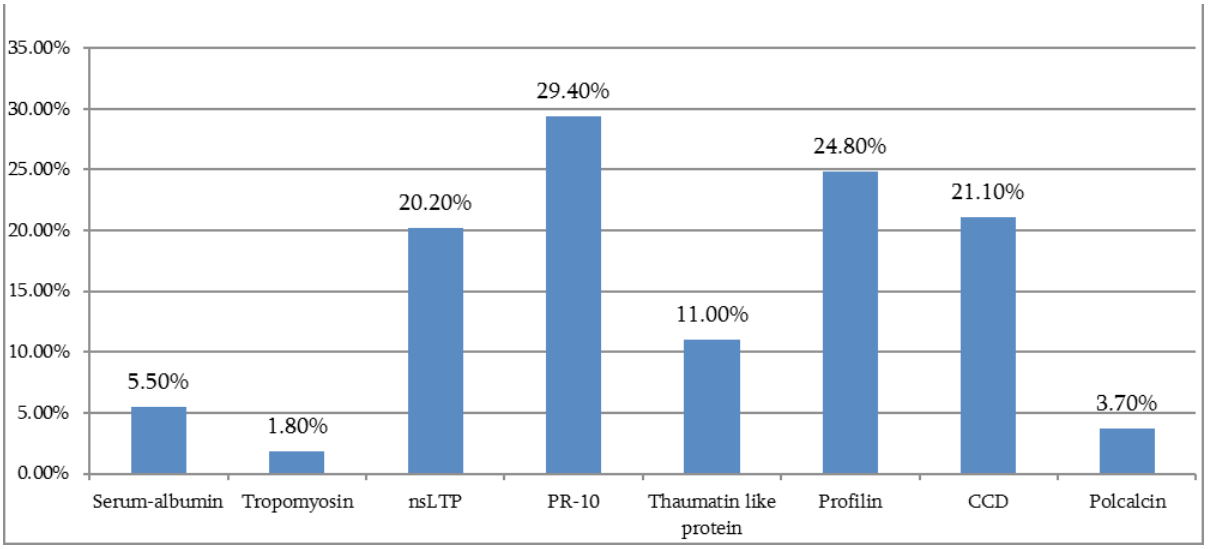

Fig. 3. Sensitization to crodd-reactive components

Table 4. Mostly co-sensitized allergen components

Grasses		
Bermuda grass	nCyn d 1	61,5%
Timothy grass	rPhl p 1	72,5%
	rPhl p 5	46,8%
Trees		
Birch	rBet v 1	27,5%
Japanese cedar	nCry j 1	78,9%
Plane tree	rPla a 1	7,3%
	nPla a 2	32,1%
Weeds		
Ragweed	nAmb a 1	48,6%
Mugwort	nArt v 1	20,2%

The cypress positive patients were mostly co-sensitized to plant allergen components: grasses (nCyn d1- 61.5% of ISAC positive cases, rPhl p 1 -72.5%, rPhl p 5 – 46.8%), trees (rBet v 1 – 27.5%, nCry j 1 – 78.9%, nPla a 2 – 32.1%) and weeds (nAmb a 1 – 48.6%, nArt v 1 – 20.2%) (Table 4).

Allergy to *Cupressaceae* pollen is a worldwide pollinosis caused by several species. The epidemiological data are alarming: in Montpellier, southern France, 19.5% of tested patients reacted positively to the *Cupressaceae* skin test, whereas in Italy and Spain, in some areas, the cypress pollen allergy affected approximately 30% of the allergic population. In an Israeli survey, 24 to 32% of patients attending an allergy clinic had an allergy to cypress pollen [8]. In a larger Italian study from Rome, 23,077 outpatient sera were studied and 42.7% of the subjects exhibited specific IgEs against cypress pollen [3]. In the present study, we show that 37.2% of patients referred to our clinic and suspected to have specific IgE to cypress pollen present sensitization. Moreover, we observed that most of the patients (90.8%) sensitized to cypress pollen were symptomatic during the cypress pollen season. We found that nasal and ocular were the most prevalent symptoms studied patients; asthma like symptoms was less frequent in this group, consistent with the findings of other studies [9]. This observation is probably due to the large aerodynamic size of cypress pollen grains (20-30 μm), which do not reach the lower airway, as they become trapped in the nasal or nasopharyngeal mucous membranes, thus causing mainly rhinitis and conjunctivitis [3].

Cupressaceae pollen frequently predominates in the winter period, but is also present throughout the remainder of the year. *Cupressaceae/Taxaceae* pollen is one of the 12 most abundant aeroallergenic pollens in Europe. Cypress pollen accounts for 40% of the total annual pollen counts in Marseille, in southern France, 38% in Antalya, and 35% in Istanbul, Turkey, 25% in Thessaloniki, Greece, 23 and 24% in Toledo and Cuenca, Spain, 18% in Nicosia, Cyprus, 17% in Palma de Mallorca, Balearic Islands, Spain, and 14%in Nerja, southern Spain. *Cupressaceae* pollen is also abundant or present outside of the Mediterranean region [3]. High cypress pollen count has been observed in our study, which accounts almost half (48.7%) of the total annual pollen counts in Tbilisi (capital of the country). The *Cupressaceae* main pollen season in Tbilisi occurs during the winter-early spring months, beginning, most of years, in January and ending in the beginning of April, in line to what has been reported in other studies [5]. High sensitization to cypress pollen among patients in our study could be explained by high exposure to *Cupressaceae* pollen, particularly - high pollen burden (48.7%), high peak pollen concentration (2811 p/m^3) and long duration of the season (145 days).

An aspect to be highlighted is that *Cupressus* sensitization is commonly associated with poly-sensitization. This phenomenon warrants a more in-depth investigation, not only to establish a correlation with other non-taxonomically related pollen species, but also to elucidate the mechanism involved in the origin of this sensitization or the responsible allergens [6]. A very interesting finding of our study was that all of the cypress-sensitized patients showed positive reactions to different allergens, especially other pollens components. 78.9% of patients sensitized to nCup a 1 were sensitized to nCry j 1, which is consistent with the high cross-reactivity reported between both allergens. It well known that there is high cross-reactivity within the cypress family, as well as cross-reactivity to mugwort and ragweed also been described [10]. High co-sensitization to Bermuda and Thimoty grass components, among studied patients, could be affected by CCD recognition, as natural (n) Cyn d 1, nPhl p 4, like nApi g 5, nCup a1, and MUXF3, express cross-reactive carbohydrate determinants (CCDs), which could lead in principal to nonspecific IgE binding. According to our data MUXF3 were positive 21.1% of cypress sensitized patients. In polysensitized patients, cypress sensitization could be the result of IgE cross-reactivity to proteins/epitopes with structures similar to those of allergens. IgE antibodies against a given allergen may bind to homologous molecules of panallergens (profilin, calcium-binding protein, lipid transfer protein, thaumatin-like protein) in different plant species [9]. In our study we observed low or moderate co-sensitization with panallergens, thus the poly-sensitization could not be explained only by cross-reactivity, especially in regards to high co-sensitization with recombinant allergens as rPhl p 1 (72,5%) and rPhl p 5 (46,8%). Futher investigations are required to clarify cross-reactivity and co-sensitization issues.

Our study has some limitations. The present study does not have an epidemiological value, as it was not carried out on the general population, but it shows the importance of cypress pollen allergy for Georgian population. In the frame of evaluation of the co-sensitization, since we relied on results from ISAC112, our analysis is restricted to the allergens covered by this procedure.

In conclusion, our data show that in Georgia, the prevalence of sensitization to cypress pollen in patients attending the allergy clinic is high (every third patient). The clinical symptoms predominantly associated with allergic rhinitis and atopic conjunctivitis, in most cases which are expressing by acute sneezing and ocular itching during the pollen season and all patients are poly-sensitized. This was the first study to give a detailed description of the clinical and molecular characteristics of cypress pollen allergic patients in Georgia.

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SUMMARY

CYPRESS POLLEN SESITIZATION IN GEORGIA: CLINICAL AND MOLECULAR CHARACTERISTICS

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Cypress pollen allergy is a widely distributed, highly prevalent and severe winter pollinosis that may be caused by several *Cupresaceae* species around the Mediterranean basin, in North America and Asia. Exposure to cypress pollen has increased steadily over the last few decades and the prevalence of allergy to cypress pollen has also dramatically increased from 0.6% to 9.8% in the general population and from 9% to 35% in allergic patients, probably because of the allergen load has become more intense.

The objective of the study was to evaluate cypress pollen allergy in Georgia and describe clinical characteristics and the molecular profile of sensitization. Patients attended to allergy clinic with suspected cypress pollen allergy (n=492) were included in the study. Diagnostic workup was performed according to local guidelines, specific IgE antibody against cypress allergen was performed using ImmunoCAP and ISAC assay platform. The airborne pollen monitoring was performed with a Burkard Seven Day Volumetric Spore-trap (Burkard Manufacturing Co Ltd, UK) during the seasons of 2019-2020, following the recommendations of European Aerobiology Society. 37.2% of studied cases were positive to cypress diagnostic test. From cypress positive 183 patients only 17 patients (9.2%) had no symptoms during the cypress season. The most frequent symptoms were sneezing and ocular itching (82.9% of patients for both symptoms), rhinorrhea was reported in 77.7% of cases and

dry cough and shortness of breath - only in 22.3% (each symptoms). More than half of frequent symptoms were reported as severe. The cypress positive patients were mostly co-sensitized to plant allergen components: grasses (nCyn d1- 61.5% of ISAC positive cases, rPhl p 1 -72.5%, rPhl p 5 – 46.8%), trees (rBet v 1 – 27.5%, nCry j 1 – 78.9%, nPla a 2 – 32.1%) and weeds (nAmb a 1 – 48.6%, nArt v 1 – 20.2%).

Our data show that in Georgia, the prevalence of sensitization to cypress pollen in patients attending the allergy clinic is high (every third patient). The clinical symptoms predominantly associated with allergic rhinitis and atopic conjunctivitis, in most cases which are expressing by acute sneezing and ocular itching during the pollen season and all patients are poly-sensitized. This was the first study to give a detailed description of the clinical characteristics of cypress pollen allergic patients in Georgia.

Keywords: cypress, allergy, sensitization

РЕЗЮМЕ

СЕНСИБИЛИЗАЦИЯ К ПЫЛЬЦЕ КИПАРИСА В ГРУЗИИ: КЛИНИЧЕСКАЯ И МОЛЕКУЛЯРНАЯ ХАРАКТЕРИСТИКА

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Аллергия к пыльце кипариса является весьма распространенным острым зимним полинозом, вызванным различными видами Cupresaceae в странах Средиземноморья, Северной Америки и Азии. В течение последних нескольких десятилетий распространенность аллергии к пыльце кипариса резко возросла (с 0,6% до 9,8%) среди населения в целом и у пациентов с аллергией (с 9% до 35%), по всей вероятности, ввиду более интенсивной экспозиции аллергена.

Целью исследования явилась оценка показателей распространенности аллергии к пыльце кипариса в Грузии и определение ее клинических характеристик и молекулярного профиля сенсибилизации.

В исследование включены пациенты, обратившиеся в аллергологическую клинику с подозрением на аллергию к пыльце кипариса (n=492). Диагностическое обследование проводилось в соответствии с местными инструкциями, специфические антитела IgE к аллергену кипариса определялись с использованием платформы ImmunoCAP и ISAC. Мониторинг переносимой по воздуху пыльцы проводился с использованием аппарата Burkard (Burkard Manufacturing, Англия) в течение сезонов 2019-2020 гг. в соответствии с рекомендациями Европейского Аэробиологического Общества.

37,2% изученных случаев дали положительный результат на диагностический тест кипариса. Из 183 позитивных к кипарису пациентов только 17 (9,2%) не имели симптомов в течение сезона цветения кипариса. Наиболее частыми симптомами были чихание и зуд вокруг глаз (82,9% пациентов по обоим симптомам), ринорея зарегистрирована в 77,7% случаев, сухой кашель и одышка - в 22,3%. Более половины частых симптомов были тяжелыми. Пациенты с положительной реакцией на кипарис в большинстве случаев были сенсибилизированы к компонентам растительных аллергенов: травы (nCyn d1 - 61,5% случаев с положительным результатом ISAC, rPhl p 1 - 72,5%, rPhl p 5 - 46,8%), деревья (rBet v 1 - 27,5%, nCry j 1 - 78,9%, nPla a 2 - 32,1%) и сорняки (nAmb a 1 - 48,6%, nArt v 1 - 20,2%). Полученные в результате исследования данные выявили высокую (каждый третий пациент с аллергией) распространенность аллергии к пыльце кипариса у исследуемых пациентов. Клинические симптомы, связанные с аллергическим ринитом и атопическим конъюнктивитом, в большинстве случаев проявляются в частом чихании и зуде вокруг глаз во время сезона цветения кипариса, все пациенты полисенсибилизированы.

Проведенное исследование является первой попыткой подробного описания клинических и молекулярных характеристик пациентов с аллергией на пыльцу кипариса в Грузии.

რეზიუმე

საქართველოში კვიპაროსის ყვავილის მტვრის მარცვლის მიმართ სენსიბილიზაცია: კლინიკური და მოლეკულური მახასიათებლები

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ალერგიისა და იმუნოლოგიის კვლევითი ცენტრი, თბილისი, საქართველო

კვიპაროსის ყვავილის მტვრის მარცვლის მიმართ ალერგია საკმაოდ გავრცელებული, მაღალი პრევალენსის მქონე ზამთრის მძიმე პოლინოზია. ბოლო ათწლეულების მანძილზე ძალიან გაიზარდა კვიპაროსის ყვავილის მტვრის მარცვლის ექსპოზიცია და, შესაბამისად, დრამატულად იმატა (0.6% დან 9.8% ზოგად პოპულაციაში და 9% დან 35% ალერგიულ პაციენტებში) მის მიმართ ალერგიის შემთხვევებმა.

კვლევის მიზანს წარმოადგენდა კვიპაროსის მიმართ ალერგიის შემთხვევების და მისი კლინიკური და მოლეკულური მახასიათებლების შესწავლა საქართველოში.

კვლევაში ჩართული იყო ალერგიის ცენტრის 492 პაციენტი, რომლებთანაც სავარაუდო იყო კვიპაროსის მიმართ ალერგია. სადიაგნოსტიკოდ გამოყენებულია ImmunoCAP და ISAC პლატფორმა. ყვავილის მტვრის მარცვლის მონიტორინგი განხორციელდა მცენარეული მტვრის მარცვლის შემაგროვებელი აპარატით (Burkard 7-day sampler) 2019-2020 წწ. ევროპის აერობიოლოგიური საზოგადოების მოთხოვნების შესაბამისად. კვიპაროსის სადიაგნოსტიკო ტესტი დადებითი აღმოჩნდა შესწავლილი პაციენტების 37,2%-ში. პოზიტიური 183 პაციენტიდან მხოლოდ 17 (9.2%) პაციენტს არ აღენიშნა სიმპტომები კვიპაროსის ყვავილობის სეზონზე. ყველაზე გავრცელებულ სიმპტომებს განეკუთვნებოდა ცემინება და თვალის ქავილი (82.9% ორივე სიმპტომისთვის), რინორეა აღინიშნებოდა 77.7%-ში, ხოლო მშრალი ხველა და სუნთქვის უკმარისობა - 22.3%-ში. სიმპტომების უმეტესობა იყო მძიმე მიმდინარეობის. კვიპაროსის მიმართ ალერგიულ პაციენტებში უმეტესად აღინიშნებოდა კო-სენსიბილიზაცია მცენარეული ალერგენული კომპონენტების მიმართ: ბალახები (nCyn d1- 61.5% ISAC დადებითი შემთხვევების, rPhl p 1 -72.5%, rPhl p 5 – 46.8%), ხეები (rBet v 1 – 27.5%, nCry j 1 – 78.9%, nPla a 2 – 32.1%) და სარეველები (nAmb a 1 – 48.6%, nArt v 1 – 20.2%).

ჩატარებულმა კვლევამ ალერგიის მქონე პაციენტებში გამოავლინა კვიპაროსის მიმართ სენსიბილიზაციის მაღალი პრევალენტობა საქართველოში (ყოველი მესამე პაციენტი). სიმპტომები უპირატესად ასოცირებული იყო ალერგიულ რინიტთან და ატოპიურ კონიუნქტივიტთან, კერძოთ მწვავე ცემინებითა და თვალების ქავილით კვიპაროსის ყვავილობის სეზონის დროს. ყველასთვის დამახასიათებელი იყო პოლისენსიბილიზაცია. საქართველოში ეს იყო პირველი კვლევა, რომელიც აღწერს კვიპაროსის მიმართ ალერგიულ პაციენტებში კლინიკურ და მოლეკულურ მახასიათებლებს.

ОСОБЕННОСТИ МОЗГОВОЙ ГЕМОДИНАМИКИ У ПАЦИЕНТОВ С СИНДРОМОМ ХРОНИЧЕСКОЙ ЦЕРЕБРАЛЬНОЙ ВЕНОЗНОЙ ДИСФУНКЦИИ И РАЗНЫМ УРОВНЕМ АРТЕРИАЛЬНОГО ДАВЛЕНИЯ

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Актуальность исследования венозной патологии головного мозга определяется не только ее большим распространением (более 80% пациентов с артериальной гипертензией и атеросклеротическим поражением сосудов мозга имеют признаки нарушения венозного оттока), но и отсутствием определенных критериев диагностики наряду с недостатками терапевтического подхода. В большинстве клинических наблюдений доминируют нарушения артериальной гемодинамики, которые сопровождаются венозной дисциркуляцией, однако в ряде случаев нарушения интракраниального венозного кровообращения преобладают над артериальной недостаточностью. Неполноценность венозной гемодинамики и артериальная недостаточность часто сочетаются в различных соотношениях. Степень компенсации венозной дисгемии зависит от возможностей коллатерального кровообращения и скорости развития интракраниального венозного застоя [1-5]. Недооценка состояния венозного звена мозгового кровообращения препятствует правильному пониманию патогенеза и клинической картины хронической церебральной ишемии. Сложность верификации нарушений венозного мозгового кровообращения является причиной ложного представления неврологов о неполноценности венозной дисгемии в патогенезе дисциркуляторной энцефалопатии [4-9].

Верификация церебральной венозной дисгемии, кроме клинической диагностики, неоднозначна, поскольку степень визуализации интракраниального и экстракраниального венозного кровотоков и трактовка параметров нередко противоречивы. При транскраниальном дуплексном сканировании хорошо визуализируются базальные вены мозга (вены Розенталя), которые являются притоками большой мозговой вены (вена Галена). Допплерографическое исследование позволяет оценить характер кровотока в базальных венах мозга и измерить его линейную скорость. В отличие от вены Розенталя большинство глубоких вен мозга весьма вариабельны и не всегда доступны к локации [5-11]. Исходя из вышеизложенного, состояние кровотока в базальных венах Розенталя (локация через височное окно) является весьма информативным показателем церебрального венозного кровотока [2]. Особенно интересным является сравнительное определение изменений церебрального кровотока у лиц с различным уровнем артериального давления (АД) с использованием стандартной методики ультразвукового исследования [8-14].

Цель исследования - сравнительный анализ особенностей церебрального кровотока у лиц с клиническими признаками синдрома хронической церебральной венозной дисфункции и различными уровнями артериального давления.

Материал и методы. За 2016-2019 гг. на базе Коммунального некоммерческого предприятия «Консультативно-диагностический центр» Святошинского района г. Киева обследовано 104 (82 женщины и 22 мужчины, средний возраст 53,60±10,27 л.) пациента, которым проведено ультразвуковое дуплексное сканирование (УЗДС) головы и шеи.

Основную группу (ОГ) составили 78 больных с клиническими признаками синдрома хронической церебральной венозной дисфункции (СХЦВД) и различным уровнем артериального давления (АД), которые распределены на 3 клинические группы, 33 - пациенты с повышенным уровнем АД (157,2±12,2/98,3±4,2) - гипертоники; 24 - с лабильным АД с преимущественно нормальными средними цифрами АД (125,23±12,2/82,22±4,14) - условные нормотоники; 21 - с пониженным АД (100,32±7,23/65,45±6,4) - гипотоники.

Контрольную группу составили 26 пациентов без клинических признаков СХЦВД и различным уровнем АД, которые распределены по такому же принципу: гипертоники - 8, нормотоники - 13, гипотоники - 5 пациентов.

Всем пациентам проведено клинико-неврологическое обследование с детализацией жалоб и анамнеза, анализ амбулаторных карт. Кровоток исследовали по стандартной методике с двух сторон во внешней сонной (ВнешСА), внутренней сонной (ВнутСА), переднемозговой (ПМА), заднемозговой (ЗМА), позвоночной артерии на уровне 2 позвонка (ХА2), позвоночной артерии на уровне 4 позвонка (ХА4), базилярной артерии (БА) и вене Розенталя (ВР). Измерялась средняя систолическая скорость кровотока, индекс резистентности [8-14].

Статистическую обработку полученных результатов осуществляли с помощью пакета прикладных программ Medstat. Статистическое сравнение проводилось с помощью точного критерия Фишера.

Результаты и обсуждение. Результаты обследования показали, что скоростные показатели церебрального кровотока с разной степенью достоверности отличались в основной и контрольной группах и изменялись в зависимости от уровня АД (таблица 1, рис.1, 2, 3).

Среди гипотоников ОГ скоростные показатели кровотока в артериях были достоверно ниже по СМА справа (p=0,0053), слева (p=0,0009); в ПМА справа (p=0,0002), слева (p=0,0000); в ЗМА справа и слева (p=0,0000;) во ВнутСА справа (p=0,0018), слева (p=0,0026); во ВнешСА справа (p=0,0062), слева (p=0,0007); в вене Розенталя были достоверно выше в сравнении с показателями группы контроля слева (p=0,0280) (таблица 1, рис.1).

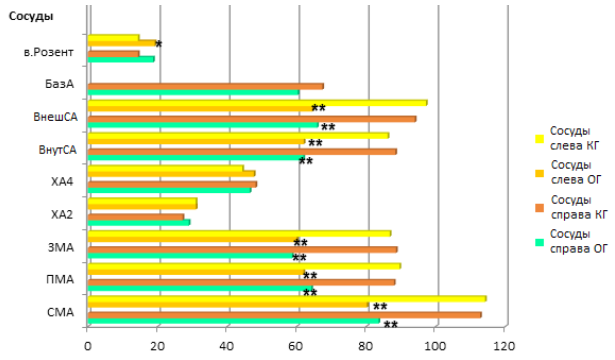

Рис. 1. Сравнение скоростей кровотока в сосудах одноименных локаций среди гипотоников основной и контрольной групп

**- достоверное различие (p<0,05) между показателями обеих групп; ** - достоверное различие (p<0,01) между показателями обеих групп*

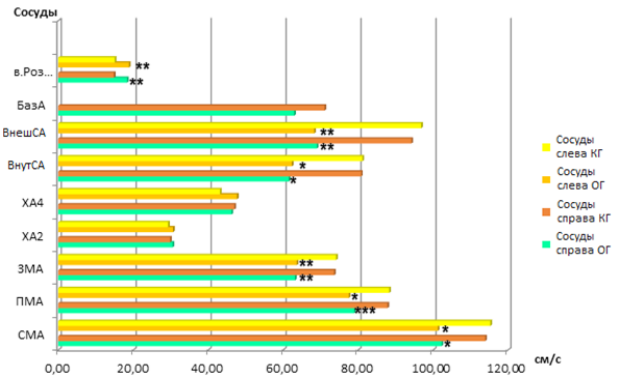

Рис. 2. Показатели средних линейных скоростей кровотока в сосудах одноименных локаций в группе условных нормотоников с СХЦВД и контрольной группе

** - достоверное различие (p<0,05) между показателями обеих групп; ** - достоверое различие (p<0,01) между показателями обеих групп; *** - тенденция (p<0,1) между показателями обеих групп (различие в клинических признаках без статистически значимых результатов)*

Среди условных нормотоников в ОГ в сравнении с контрольной группой показатели скорости в СМА справа и слева были достоверно ниже (p<0,05); в ПМА справа на уровне тенденции (p<0,1), а слева достоверно понижены (p<0,05); в ЗМА достоверно снижены справа и слева (p<0,01); во ВнутСА достоверно ниже справа (p<0,001) и слева (p<0,01); во ВнешСА - справа (p<0,001) и слева (p<0,0001). Среди показателей скоростей в вене Розенталя достоверно повышенные скорости в ОГ против показателей группы сравнения - справа (p<0,05), и слева (p<0,01) (таблица1, рис. 2). Наличие клинических признаков СХЦВД у пациентов с лабильным АД сопровождается достоверным замедлением в большинстве церебральных артерий и достоверным ускорением в вене Розенталя скорости кровотока в сравнении с больными с таким же АД и без клинических проявлений церебральной венозной дисфункции.

В основной группе гипертоников наблюдалось меньше расхождений с группой сравнения: достоверно ниже были скоростные показатели в СМА справа (p<0,05), на уровне тенденции в СМА слева (p<0,1); в ЗМА слева снижены на уровне тенденции (p<0,1). Следует отметить, что у пациентов с повышенным АД, в отличие от больных с другими уровнями АД, наблюдалось статистически значимое ускорение линейной скорости кровотока в ХА2 (p<0,05). Достоверное повышение средней линейной скорости кровотока наблюдалось в основной группе в вене Розенталя справа и слева (p<0,05) (таблица 1, рис. 3).

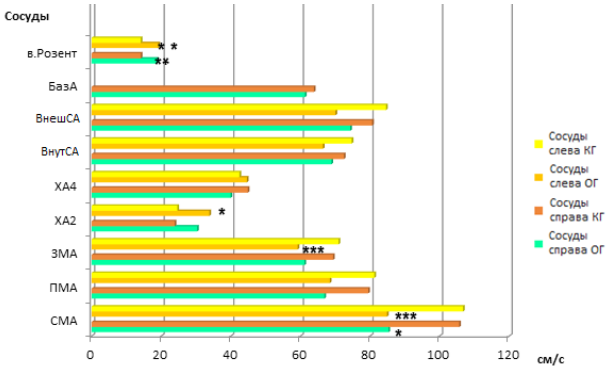

Рис. 3. Показатели средних скоростей кровотока в сосудах одноименных локаций в группе гипертоников с СХЦВД и контрольной группе

** - достоверное различие (p<0,05) между показателями обеих групп; ** - достоверное различие (p <0,01) между показателями обеих групп; *** - тенденция (p <0,1) между показателями обеих групп(различие в клинических признаках без статистически значимых результатов)*

Из предыдущих диаграмм явствует, что у пациентов с клиническими признаками СХЦВД в отличие от контрольной группы наблюдалось достоверное замедление кровотока в различных артериях и ускорение в венах Розенталя. Достоверное различие скоростных показателей характеризовалось различным проявлением в зависимости от уровня АД. Неожиданностью явилось минимальное отличие показателей среди гипертоников основной и контрольной групп, что, по всей вероятности, свидетельствует о субклиническом течении венозной дисциркуляции, что и подтвердилось УЗДС. Полученные результаты диктуют необходимость установить различие скоростей кровотока у трех клинических подгрупп ОГ (гипертоники, нормотоники и гипотоники), таблица 2.

Таблица 1. Сравнение средних показателей скоростей кровотока по сосудам одноименных локаций в подгруппах ОГ с подгруппами контрольной группы

Сосуды	**Гипотоники**		**Усл. Нормотоники**		**Гипертоники**	
	Правая	Левая	Правая	Левая	Правая	Левая
	p-level Стьюдент	p-level Стьюдент	p-level Стьюдент	p-level Стьюдент	p-level Стьюдент	p-level Стьюдент
СМА	0,0053	0,0009	0,0318	0,0250	0,0491	0,0534
ПМА	0,0002	0,0000	0,0572	0,0253	0,1611	0,1771
ЗМА	0,0000	0,0000	0,0071	0,0057	0,2378	0,0760
ХА2	0,7616	0,9938	0,8187	0,6556	0,1356	0,0430
ХА4	0,7969	0,5742	0,7497	0,1399	0,1958	0,6349
ВнутСА	0,0018	0,0026	0,0002	0,0010	0,6968	0,4091
ВнешСА	0,0062	0,0007	0,0010	0,0000	0,5256	0,1073
БазА	0,4127		0,5560		0,7179	
Вена Розент	0,5590	0,0280	0,0114	0,0077	0,0125	0,0117

Таблица 2. Сравнение скоростных характеристик одноименных локаций в подгруппах ОГ с различными показателями АД (условные нормотоники/гипотоники, условные нормотоники/гипертоники, гипотоники/гипертоники)

Усл.нормотоники/гипотоники						
Сосуды	**Правые локации сосудов, см/с**			**Левые локации сосудов, см/с**		
	нормотоники (n=24)	гипотоники (n=21)	p	нормотоники (n=24)	гипотоники (n=21)	p
Вена Розент	18,35	18,75	0,762357	18,88	19,21	0,802904
СМА	102,29	83,57	0,0026	101,29	80,24	0,001316
ПМА	78,96	64,24	0,000317	77,54	62	0,00015
ЗМА	63,17	58,71	0,229303	63,63	60,05	0,292934
ХА2	30,5	29	0,648321	30,63	31,05	0,89942
ХА4	46,22	46,52	0,930078	47,7	47,71	0,995714
ВнутСА	61,5	61,95	0,925957	62,33	62,05	0,947739
ВнешСА	69	65,9	0,59481	68,29	64,52	0,457031
Усл.нормотоники/гипертоники						
Сосуды	*Правые локации сосудов, см/с*			*Левые локации сосудов, см/с*		
	нормотоники (n=24)	гипотоники (n=21)	p	нормотоники (n=24)	гипотоники (n=21)	p
Вена Розент	18,35	18,81	0,709296	18,88	19,16	0,830022
СМА	102,29	85,32	0,009386	101,29	84,88	0,02395
ПМА	78,96	66,91	0,032791	77,54	68,46	0,113338
ЗМА	63,17	61,18	0,630944	63,63	59,21	0,24618
ХА2	30,5	30,32	0,950546	30,63	33,85	0,274402
ХА4	46,22	39,97	0,01657	47,7	44,61	0,318328
ВнутСА	61,5	68,91	0,191862	62,33	66,47	0,465252
ВнешСА	69	74,33	0,36271	68,29	70,09	0,718145
Гипотоники/Гипертоники						
	Правые локации сосудов, см/с			*Левые локации сосудов, см/с*		
	гипотоники (n=21)	гипертоники (n=33)	p	гипотоники (n=21)	гипертоники (n=33	p
Вена Розент	18,75	18,81	0,962573	19,21	19,16	0,972201
СМА	83,57	85,32	0,799159	80,24	84,88	0,528335
ПМА	64,24	66,91	0,636932	62	68,46	0,263856

ЗМА	58,71	61,18	0,575888	60,05	59,21	0,833155
ХА2	29	30,32	0,691115	31,05	33,85	0,400812
ХА4	46,52	39,97	*0,050047*	47,71	44,61	0,370856
ВнутСА	61,95	68,91	0,245546	62,05	66,47	0,47397
ВнешСА	65,9	74,33	0,188529	64,52	70,09	0,315382

Сравнение средних скоростных показателей в правых одноименных сосудах головы и шеи в группах с условно нормальными и пониженными показателями АД выявило достоверное снижение скоростей в группе гипотоников в правой СМА и правой ПМА ($p<0,01$); между группами с нормальными и повышенными показателями АД справа выявлено достоверное снижение скоростей в группе гипертоников в СМА и ПМА ($p<0,01$, $p<0,05$), в ХА4 ($p<0,05$). При сравнении показателей в группах с пониженными и повышенными показателями АД справа выявлено статистически значимое замедление кровотока у гипертоников только в ХА4 ($p<0,05$),таблицы 1 и 2.

Подобная картина выявлена при сравнении показателей в левых магистральных сосудах: в группах с условно нормальными и пониженными показателями АД наблюдалось достоверное снижение средних показателей скорости в подгруппе гипотоников в левой СМА и в левой ПМА ($p<0,01$); в сравнение группах с условно нормальными и повышенными показателями АД показало достоверное увеличение скорости в подгруппе гипертоников между артериями в левой СМА ($p<0,05$). Сравнение подгрупп ОГ со сниженными и повышенными показателями АД достоверной разницы в скоростях кровотока не выявило, что, по всей вероятности, объяснятеся стадийностью течения СХЦВД и АГ: гипотония у больных, возникшая на фоне СХЦВД, часто со временем трансформируется в гипертонию.

Следует предположить, что достоверное замедление кровотока в бассейнах ПМА и СМА при сравнении скоростных характеристик в одноименных локациях в подгруппах основной группы (гипотоники и гипертоники) в отличие от контрольной группы, объясняется при венозной дисгемии большей подверженностью этих бассейнов ишемии с клинически сопровождающимися соответствующими симптомами.

Выводы.

1. Средние показатели линейной скорости церебрального кровотока с различной степенью достоверности в зависимости от уровня артериального давления отличались у больных синдромом хронической церебральной венозной дисфункции в сравнении с таковыми контрольной группой.

2. Наличие клинических признаков СХЦВД у больных с различным уровнем АД сопровождалось достоверным замедлением кровотока в большинстве церебральных артерий и достоверным ускорением в вене Розенталя в сравнении с больными без клинических проявлений венозной дисфункции и с таким же АД. У пациентов с повышенным уровнем АД наблюдается статистически значимое ускорение линейной скорости кровотока в ХА2 ($p<0,05$).

3. В подгруппах гипертоников с СЦХВД показателей средней линейной скорости кровотока с достоверным различием было вдвое меньше, чем в контрольной группе: достоверное снижение скоростных показателей выявлено только в бассейнах СМА с двух сторон ($p<0,01$, $p<0,05$ справа и слева, соответственно) и слева в бассейнах ЗМА, ХА2; достоверное повышение кровотока отмечалось в венах Розенталя ($p<0,01$, $p<0,05$). Указанное несоответствие следует объяснить тем, что гипертоники из группы сравнения, т.е. без клинических симптомов СХЦВД, уже имеют его субклинические проявления, о чем свидетельствуют изменения показателей УЗДС. Таким образом, можно считать больных с АГ пациентами с СХЦВД.

4. Наличие достоверного замедления кровотока в одноименных локациях в подгруппах основной группы (гипотоники и гипертоники) в бассейнах ПМА и СМА, объясняется тем, что при венозной дисгемии эти бассейны являются более уязвимыми и быстрее реагируют на недостаточность венозного кровообращения.

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SUMMARY

PECULIARITIES OF CEREBRAL HEMODYNAMICS IN PATIENTS WITH CHRONIC CEREBRAL VENOUS DYSFUNCTION SYNDROME AND DIFFERENT ARTERIAL PRESSURE LEVELS

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For the purpose of research and comparative analysis of the features of cerebral blood flow, determined by the ultrasonic duplex scanning (USDS) method, in persons with clinical signs of chronic cerebral venous dysfunction syndrome and different levels of arterial pressure (AP), ultrasound of the head and neck was performed in 104 patients.

78 people - the main group (MG), where patients were selected for clinical signs of the presence of chronic cerebral venous dysfunction syndrome (CCVDS) and different blood pressure levels, were divided into 3 clinical groups: 33 people - people with high blood pressure (157.2±12,2/98.3±4.2) - hypertensive patients; 24 people with labile blood pressure with predominantly normal average blood pressure (125.23±12.2/82.22±4.14) - conditional normotonics; 21 people with low blood pressure (100.32±7.23/65.45±6.4) were hypotonic. Also, 26 patients of the comparison group were recruited - people without clinical signs of CCVDS and different levels of blood pressure, distributed according to the same principle: hypertensive patients - 8 people, normotonic patients - 13 people, hypotensive patients - 5 people. The total age of the patients ranged from 35 to 65 years (mean age 53.60±10.27 years). Among the patients there were 82 women and 22 men. Statistical comparison was performed using Fisher's exact test.

The mean velocity indices of cerebral blood flow with varying degrees of reliability, depending on the level of arterial pressure, differed in patients with chronic cerebral venous dysfunction syndrome versus the indices of the comparison groups. The presence of clinical signs of CCVDS in people with different blood pressure levels was accompanied by a significant slowdown in blood flow in most cerebral arteries and a significant acceleration in the Rosenthal vein compared with patients without clinical manifestations of venous dysfunction and with the same arterial pressure. Attention was drawn to the fact that in persons with increased blood pressure, in contrast to the rest of the patients, a statistically significant acceleration of the linear blood flow velocity in vertebral artery at the level of the second cervical vertebra (VA2) was observed ($p<0.05$).

In the subgroups of hypertensive patients with CCVDS, the mean linear blood flow velocity with a significant difference was twice less than the comparison group: a significant decrease in velocity parameters was only in the middle cerebral arteriasis (MCA) basins on both sides ($p<0.01$, $p<0.05$ on the right and left, respectively) and on the left in the pools posterior cerebral artery (PCA), VA2, a significant increase in blood flow ($p<0.01$, $p<0.05$) - in the veins of Rosenthal (VR). This discrepancy can be explained by the fact that hypertensive patients from the comparison group, that is, without clinical symptoms of CCVDS, already have subclinical manifestations of CCVDS, as evidenced by changes in the USDS indicators.

Keywords: chronic cerebral venous dysfunction syndrome, CCVDS, arterial pressure, AP, USDS, mean linear blood flow velocity, cerebral hemodynamics.

РЕЗЮМЕ

ОСОБЕННОСТИ МОЗГОВОЙ ГЕМОДИНАМИКИ У ПАЦИЕНТОВ С СИНДРОМОМ ХРОНИЧЕСКОЙ ЦЕРЕБРАЛЬНОЙ ВЕНОЗНОЙ ДИСФУНКЦИИ И РАЗНЫМ УРОВНЕМ АРТЕРИАЛЬНОГО ДАВЛЕНИЯ

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Цель исследования - сравнительный анализ особенностей церебрального кровотока у лиц с клиническими признаками синдрома хронической церебральной венозной дисфункции и различными уровнями артериального давления.

104 пациентам (82 женщины и 22 мужчины) проведено ультразвуковое дуплексное сканирование (УЗДС) головы и шеи. Основную группу (ОГ) составили 78 больных с клиническими признаками синдрома хронической церебральной венозной дисфункции (СХЦВД) и различным уровнем артериального давления (АД). Больные распределены на 3 клинические группы, 33 - с повышенным уровнем АД (157,2±12,2/98,3±4,2) - гипертоники; 24 - с лабильным АД с нормальными средними цифрами АД (125,23±12,2/82,22±4,14) - условные нормотоники; 21 больной - с пониженным АД (100,32±7,23/65,45±6,4) - гипотоники. Группу сравнения составили 26 пациентов без клинических признаков СХЦВД и с различным уровнем АД, которые распределены по такому же принципу: гипертоники - 8, нормотоники - 13, гипотоники - 5 пациентов. Возраст пациентов варьировал в пределах от 35 до 65 лет (средний возраст 53,60±10,27 г.). Статистическое сравнение проводилось по точному критерию Фишера.

Средние скоростные показатели церебрального кровотока с разной степенью достоверности в зависимости от уровня АД отличались у больных СХЦВД от показателей группы сравнения. Наличие клинических признаков СХЦВД у лиц с различным уровнем АД сопровождалось достоверным замедлением кровотока в большинстве церебральных артерий и достоверным ускорением в вене Розенталя в сравнении с больными без клинических проявлений венозной дисфункции и с таким же АД. У лиц с повышенным АД, в отличие от остальных больных, наблюдалось статистически значимое ускорение линейной скорости кровотока в позвоночной артерии на уровне 2 шейного позвонка (ХА2) ($p<0,05$).

В подгруппах гипертоников с СХЦВД показателей средней линейной скорости кровотока с достоверным различием было вдвое меньше, чем в группе сравнения: достоверное снижение скоростных показателей выявлено только в бассейнах среднемозговой артерии с двух сторон ($p<0,01$, $p<0,05$ справа и слева, соответственно), слева в бассейнах заднемозговой артерии, ХА2 и достоверное повышение кровотока в венах Розенталя ($p<0,01$, $p<0,05$). Указанное несоответствие следует объяснить тем, что гипертоники из группы сравнения, т.е. без клинических симптомов СХЦВД, уже имеют его субклинические проявления, о чем свидетельствуют изменения показателей УЗДС.

რეზიუმე

ცერებრული ჰემოდინამიკის თავისებურებები პაციენტებში ქრონიკული ცერებრული ვენური დისფუნქციით და არტერიული წნევის სხვადასხვა დონით

ნ.პრიტიკო, ო.კოვალენკო

სახელმწიფო სამეცნიერო დაწესებულება "პრევენციული და კლინიკური მედიცინის სამეცნიერო-პრაქტიკული ცენტრი"; პ. შუპიკის სახ. ეროვნული დიპლომისშემდგომი განათლების სამედიცინო აკადემია, კიევი; კომუნალური არაკომერციული საწარმო "საკონსულტაციო და დიაგნოსტიკური ცენტრი" სვიატოშინსკის რაიონი, კიევი, უკრაინა

კვლევის მიზანს წარმოადგენდა ცერებრული სისხლის ნაკადის თავისებურებების შედარებითი ანალიზი პაციენტებში ქრონიკული ცერებრული ვენური უკმარისობის კლინიკური ნიშნებით და არტერიული წნევის სხვადასხვა დონით.

104 პაციენტს (82 ქალი, 22 მამაკაცი) ჩაუტარდა თავის და კისრის ულტრაბგერითი დუპლექს-სკანირება. ძირითადი ჯგუფი შეადგინა 78 პაციენტმა ქრონიკული ცერებრული ვენური უკმარისობის კლინიკური ნიშნებით და არტერიული წნევის სხვადასხვა დონით, რომელნიც დაიყო 3 კლინიკურ ჯგუფად: 33 – არტერიული წნევის მომატებული დონით (157,2±12,2/98,3±4,2) - ჰიპერტონიკები, 24 – არტერიული წნევის უმეტესად ნორმალური მაჩვენებლებით (125,23±12,2/82,22±4,14) - პირობითი ნორმოტონიკები, 21 – დაქვეითებული არტერიული წნევით (100,32±7,23/65,45±6,4) - პირობითი ჰიპოტონიკები.

შედარების ჯგუფი შეადგინა 26 პაციენტმა ქრონიკული ცერებრული ვენური უკმარისობის კლინიკური ნიშნების გარეშე და არტერიული წნევის სხვადასხვა დონით, რომელნიც იგივე პრინციპით დაიყო ჯგუფებად: ჰიპერტონიკები – 8, ნორმოტონიკები – 13, ჰიპოტონიკები – 5 პაციენტი. პაციენტების ასაკი ვარირებდა 35-65 წწ. ფარგლებში (საშუალო ასაკი - 53,60±10,27 წ.). სტატისტიკური შედარება ჩატარდა ფიშერის კრიტერიუმის მიხედვით.

ცერებრული ჰემოდინამიკის სიჩქარის მახასიათებლები, სარწმუნოობის სხვადასხვა ხარისხით დამოკიდებული არტერიული წნევის დონეზე, შედარების ჯგუფსა და ქრონიკული ცერებრული ვენური უკმარისობის კლინიკური ნიშნებით ჯგუფში იყო განსხვავებული. ცერებრული ვენური უკმარისობის კლინიკური ნიშნების არსებობას პირებში არტერიული წნევის სხვადასხვა დონით სარწმუნოდ სდევს თან სისხლის ნაკადის შენელება ცერებრული არტერიების უმეტესობაში და სარწმუნო მატება როზენტალის ვენაში, შედარების ჯგუფთან მიმართებით. პირებში არტერიული წნევის მომატებული მაჩვენებლებით, დანარჩენი პაციენტებისაგან განსხვავებით, აღინიშნება ხაზოვანი სიჩქარის მნიშვნელოვანი მატება ხერხემლის არტერიაში ხერხემლის II მალის დონეზე (ХА2) ($p<0,05$).

ჰიპერტონიკებში ქრონიკული ცერებრული ვენური უკმარისობის კლინიკური ნიშნებით სისხლის ხაზოვანი სიჩქარის საშუალო მაჩვენებლები სარწმუნოდ ორჯერ ნაკლებია, ვიდრე შედარების ჯგუფში; სიჩქარის მაჩვენებლების შემცირება აღინიშნა მხოლოდ ტვინის შუა არტერიის აუზში ორივე მხარეს - მარჯვნივ და მარცხნივ, შესაბამისად $p<0,01$, $p<0,05$; მარცხნივ - ტვინის უკანა არტერიის აუზში და ХА2-ში, ხოლო როზენტალის ვენებში – სარწმუნო მატება ($p<0,01$, $p<0,05$). აღნიშნული შეუსაბამობა უნდა აიხსნას იმით, რომ ჰიპერტონიკებს შედარების ჯგუფიდან, ანუ ქრონიკული ცერებრული ვენური უკმარისობის კლინიკური ნიშნების გარეშე, უკვე აქვთ მისი სუბკლინიკური გამოვლინებანი, რაზედაც მეტყველებს ულტრაბგერითი დუპლექს-სკანირების შედეგები.

BURNOUT IN MENTAL HEALTH PROFESSIONALS AND THE MEASURES TO PREVENT IT

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The American psychiatrist first studied the phenomenon of the psychological state of psychiatric workers as "emotional burnout syndrome" (EES) or "predictors of the development of emotional burnout" (EER) in the 70s of the twentieth century G. Friedenberg. PREV occurs in health professionals as a result of their professional medical activities, namely in constant contact with mentally ill patients during the work shift, which leads to depletion of their psychological and physical resources [24].

Nowadays, this phenomenon of OER has studied in other areas of professional activity. Still, in the medical field, the professional responsibilities of health professionals are associated with a high degree of responsibility for the lives and health of others. That is why it requires urgent specialist decision-making, self-discipline, ability to maintain high efficiency in extreme conditions, emotional impact, constant psychological, and intellectual stress. Working in such stressful situations affects not only the quality of medical care but also is a risk of morbidity among health professionals [16,21,23].

According to research conducted in various countries around the world, among physicians and medical staff of health care facilities, the rate of burnout varies from 31.4% to 85.8% [17,22,25,26]. It should have noted that in Ukraine, this figure is higher and ranges from 73% to 89.3% [4,5,9].

According to the literature, PREV is more common in women than in men. It should have noted that the burnout syndrome depends on age, seniority, marital status, specialty and position, microclimate both in the team and at home [1,8,9,16,25]. The leading causes of burnout are the following factors:

1. Individual factors – features of character, temperament, i.e., psychophysiological processes of the human body.
2. Factors that affect a person from the outside – the conditions of communication between colleagues, workload, working conditions, financial status (low wages, lack of housing, etc.), and others.

Given the high incidence of PER among general health professionals, the study of PER among psychiatric staff and its impact on their health is hugely relevant today, which led to the topic of our research.

The purpose of the work is to determine the manifestations and level of PER in employees of the psychiatric hospital of Vinnytsia and the development of preventive measures to prevent it.

Material and methods. The study involved 224 respondents – the medical staff of the regional psychiatric hospital in Vinnytsia. Among the subjects were women – 84.8%, and men – 15.2%. Of the total number of subjects, doctors accounted for 38.8% (87 people), nurses – 61.2% (137 people). The average age of respondents among doctors was 44.6±12.2 years, among SMP 37.2±11.4 years. Work experience in professional activities was: among doctors – 19.7±12.3 years and SMP – 15.5±11.1 years. The study used a psychodiagnostic method of emotional burnout Boyko V.V. and adapted the method of Vodopyanova N.E. [15].

Using the method of Boyko V.V., an assessment of three phases of the development of PREV has carried out: the phase of stress, resistance, and exhaustion. And also according to the results of the questionnaire "Professional burnout" (P.B.), adapted by the method of Vodopyanova N.E. identified: "emotional exhaustion", which is characterized by loss of energy, the appearance of signs of psychophysiological fatigue, signs of anxiety and depression, anger, aggression, a sense of exhaustion; "Depersonalization", which is characterized by increased psychological distancing from work, decreased empathy and cynical attitude towards others, patients, pessimistic thoughts about work; "Reduction of professional achievements", which is characterized by negative self-esteem, indifference to the performance of their professional duties and reduced professional efficiency, reduced professional motivation and self-esteem.

Statistical processing of the survey results was performed in the licensed standardized package "Statistica 6.1 for Windows" with the calculation of the arithmetic mean, standard arithmetic mean. The significance of the difference has assessed using Student's t-test (t). The content analysis of domestic and foreign scientific sources, biblio-semantic, analytical, and statistical research methods have used in work.

Results and discussion. The WHO presented the new 11th edition of the International Classification of Diseases (ICD-11) at the World Health Assembly in Geneva (2019). In this publication, the section "Factors affecting health status or contact with health care services" has been supplemented by the results of research on the treatment of healthcare professionals and other patients about the occurrence of COPD. It has recognized that this is a syndrome that occurs as a result of "chronic stress in the workplace, which the worker did not cope in time", and therefore referred to the manifestations before the disease with the following headings: "Adaptation disorders" – F43; "Burnout" – Z73.0; "Neurasthenia" – F48 [2].

Given the high rate of PER among general health professionals, we conducted research to identify CER among medical staff at the Regional Psychiatric Hospital in Vinnytsia, which was attended by 224 nurses. According to the results of our research, it has found that the degree of formation of symptoms of emotional burnout in both physicians and SMP depended on the phase of the syndrome (table 1). The average value of emotional exhaustion among physicians was – 17.5 points and among SMP – 20.2 points. PREV is more likely to occur due to insufficient positive feedback due to poor treatment outcomes, and therefore feelings of failure, guilt, and helplessness.

Analyzing the data in table 1, it can have stated that the CME stress phase has formed in every fifth medical worker; among men, SMP registered the most significant number – 22.6%.

In the second phase of CMEA "Resistance", the degree of formation is highest in men working as nurses and was 43.8%, in male doctors – 35.5%, which indicates the readiness of these contingents in the next phase of "Exhaustion". This research has evidenced by the degree of formation of the last period of "Exhaustion": the average male medical staff is high and is 39.1%, male doctors – 27.4%. In women-doctors, in the second phase of CMEA "Resistance", the degree of resistance formation sharply increases (52.0%) in comparison with male doctors (32.3%) with the transition to the category of resistance formation at the level of 36.0%, which is levels of the indicator in male doctors (35.5%).

Women working as midwives are the most psychologically resilient, and the degree of formation of the "Resistance" phase is 0%, which is confirmed by a high degree (77.8%) of resistance (immaturity) to exhaustion.

Analyzing the indicators of the formation of the phase of "Exhaustion", the most psychologically vulnerable group are men who work as midwives. This figure was 18.8%, while in all other categories it averaged 11.4% (male doctors – 11.3%, female doctors – 12.0%, female nurses – 11.1 %).

According to the results of processing and interpretation of the results according to the "key" of the CMEA questionnaire according to the method of Boyko V.V, it was found that the phase of the stress of the psycho-emotional state of health workers was associated with the actual experiences: psycho-traumatic circumstances in doctors – 14.2 points, in SMP – 14.1 points; anxiety and depression in doctors – 10.6 points, in SMP – 9.9 points; dissatisfaction with themselves in doctors – 9.5 points, in SMP – 9.2 points, which did not reveal a significant difference between these medical staff. However, the feeling of being driven into a cell in the SMP has registered at the level of 7.5 points, which was 1.3 times (5.5 points) higher than in physicians at $p<0.02$.

These results confirm the presence of emotional burnout in the medical staff of a psychiatric institution, which requires immediate implementation of psycho correction measures, creating conditions for psychological relief and systematic preventive examinations.

During the study, a survey of medical staff has conducted according to another method - the questionnaire «Professional Burnout» (P.B.) adapted by Vodopyanova N. and Starchenkova E. The questionnaire contained 22 statements about the feelings and experiences associated with the performance of work. It consists of three subscales: "Emotional Exhaustion", "Depersonalization", and "Professional Achievement". Subscale "Emotional exhaustion" is characterized by loss of energy, the appearance of signs of psychophysiological fatigue, manifestations of depression, anger, aggression, a feeling of exhaustion [15].

According to the results of the study of P.B. in the medical staff on the subscale, "Emotional Exhaustion" was established as follows. High levels of burnout in terms of emotional exhaustion were observed in women-doctors and women SMP – 19.4% and 19.5%, respectively. These indicators are 1.6 times higher than the same figure for male doctors – 12.0%. A high level of occupational burnout has registered in men with SMP, which is – 33.3%, which exceeds similar indicators of all other studied contingents: 2.7 times compared to male doctors (12.0%), 1.7 times incomparable with women-doctors and SMP women.

Among all medical workers, according to the scale score, "Very high rate" P.B. was observed in women SMP – 14.8%, in women-doctors, this figure was 4.6 times lower (3.2%). Men with a "very high rate", P.B. was not detected (Table 2).

These results have explained by the fact that women are more responsible for work, professional responsibilities, and they are more emotionally exhausted. The phase of emotional exhaustion in the severity of the leading symptoms was associated with the presence of psychosomatic and psychophysiological disorders in physicians – 8.4 points ($t=2.04$; $p<0.05$); and more pronounced in the SMP – 10.4 points. Emotional avoidance, as a component of emotional exhaustion, was observed in physicians and was rated on a scale of 10.9 points, in SMP – at 12.2 points.

Table 1. Formation of emotional burnout syndrome (by phases and degree of structure) in medical staff of a psychiatric health care institution, (in%)

SEV phases	**Voltage phase "Alarm voltage"**			**Resistance Phase**			**Depletion phase**		
The degree of formation of CMEA									
The medical staff of the CHP	Unformed (9 points or less)	Formation (10-15 point)	Formation (16 points or more)	Unformed (9 points or less)	Formation (10-15 point)	Formation (16 points or more)	Unformed (9 points or less)	Formation (10-15 point)	Formation (16 points or more)
Doctors, n=87									
Men (n=25)	48,4	32,3	19,4	32,3	32,3	35,5	61,3	27,4	11,3
Women (n=62)	52,0	28,0	20,0	12,0	52,0	36,0	50,0	48,0	12,0
Paramedics, n=137									
Men (n=9)	42,2	35,2	22,6	19,5	36,7	43,8	42,2	39,1	18,8
Women (n=128)	77,8	0	22,2	55,6	44,4	0	77,8	11,1	11,1

Table 2. Occupational burnout of medical workers on a subscale "Emotional exhaustion" depending on the profession and gender of employees, in%

The medical staff of the CHP, n		**Emotional Exhaustion Subscale**			
		Low level (5-16 points)	**Intermediate level (17-25 points)**	**High level (26-34 points)**	**Very high level (more than 34 points)**
Doctors, n=87	Men (n=25)	40%	48%	12%	0
	Women (n=62)	54,8%	22,6%	19,4%	3,2%
Paramedics n=137	Men (n=9)	44,4%	22,2%	33,3%	0
	Women (n=128)	30,5%	35,2%	19,5%	14,8%

Table 3. Occupational burnout of medical workers on a subscale " Depersonalization/Cynicism" depending on the profession and gender of employees, in%

The medical staff of the CHP, n		Subscale "Depersonalization/Cynicism"			
		Low level (1-4 points)	Intermediate level (5-9 points)	High level (10-13 points)	Very high level (more than 14 points)
Doctors n=87	Men (n=25)	8%	52%	24%	16%
	Women (n=62)	3,2%	30,6%	45,2%	21,0%
Paramedics n=137	Men (n=9)	22,2%	22,2%	22,2%	33,3%
	Women (n=128)	8,6%	21,9%	33,6%	35,9%

Table 4. Occupational burnout of medical workers on a subscale\ "Reduction of professional achievements" depending on the profession and gender of employees, in%

The medical staff of the CHP, n		Subscale "Reduction of professional achievements"			
		Low level (37-48 points)	Intermediate level (36-28 points)	High level (27-22 points)	Very high level less than 22 points)
Doctors n=87	Men (n=25)	24,0%	32,0%	16,0%	28,0%
	Women (n=62)	8,1%	45,2%	33,8%	12,9%
Paramedics n=137	Men (n=9)	0	66,7%	11,1%	22,2%
	Women (n=128)	6,25%	35,9%	31,3%	26,6%

The phase of «Depersonalization/Cynicism» P.B. has characterized by increased psychological distancing from work, decreased empathy, and cynical attitude towards others, patients, pessimistic thoughts about work. Regarding the formation of this phase of "Depersonalization/Cynicism" in medical staff on the indicator of "Exhaustion", it averaged almost the same level: doctors – 8.1 points and SMP – 8.3 points at $p \leq 0.05$.

According to table 3, depersonalization has observed in 66% of women physicians, with high and very high levels recorded in 45.2% and 21.0% of cases, respectively. Among women with SLE, this figure is slightly higher – 69.5% (high and very high levels have registered in 33.6% and 35.9% of cases, respectively). For male physicians and male SMP, these rates are 40% and 55.5%, respectively.

These results indicate a decrease in empathy and cynical attitude towards patients by 2/3 of female medical staff and every other male medical staff, which creates a stigma for the mentally ill and is a violation of moral and ethical principles of physician behavior and unacceptable.

The third stage of P.B. research concerned the definition of the phase of "Reduction of professional achievements" in medical workers, which is characterized by negative self-esteem, the emergence of employees' feelings of incompetence in their professional field, awareness of failure in it, curtailment of professional activities. Professional responsibilities, reduced professional efficiency, decreased professional motivation, and self-esteem. According to the results of research, it has established that the formation of "Reduction of professional achievements" in medical staff on the indicator of "Resistance" was due to inadequate industrial emotional response in doctors – 15.0 points and in SMP – 15.1 points; reduction of professional responsibilities of doctors – 13.7 points, SMP – 16.1 points; emotional and moral disorientation in doctors – 12.3 points, in SMP – 11.9 points. The average level of reduction of professional achievements in physicians was 28.2 points, in SMP – 25.8 points.

According to the data of table 4, the reduction of personal achievements has observed in 57.9% of SMP women, with high and very high levels have registered in 31.3% and 26.6% of cases, respectively. Among women doctors, this figure is slightly lower – 46.7% (tall and very high levels have registered in 33.8% and 12.9% of cases, respectively). In male doctors and SMP men, these figures are lower and are at 42% and 33.3%, respectively.

Based on the study, we noted an unusually high degree of formation of PREV in SMP than in physicians due to their constant physical contact with patients and, therefore, the continual action of psycho-traumatic factors at work. These include unsatisfactory working conditions, high responsibility, and workload, long work shifts, aggression from patients and their relatives, low wages.

According to the calculations of the integrated burnout rate, the high level was SMP for women – 43.8% for male doctors – 40.0% and for women doctors – 37.1%. To assess the integrated indicator of the subscale, the following are several degrees – 3-4 points; average degree – 5-6 points; high degree – 7-9 points; very high – more than 9 points [15]. According to our data, the average score for doctors was 7.3 points and for SMP – 8.1 points, which has estimated as a high level of emotional burnout, which is comparable to the results of many other authors [9,11,13,14,19].

Therefore, it is necessary to continually monitor the medical teams for timely identification of the causes of the formation of PER and P.B., preventive measures to minimize them and prevent pre-disease conditions. Studies have also shown that stress, emotional exhaustion, signs of anxiety, and depression in health care workers can lead to reduced job satisfaction, teamwork, and personal problems that can occur [18]. According to a survey of medical staff in hospitals in South Bohemia, 79.0% of respondents considered it necessary to carry out preventive measures against PER [6]. Both sophisticated and individual pieces of training, training for prevention, and treatment of already formed PREV at medical workers have offered. Instructions on communication and assertiveness showed a positive result in reducing the level of signs of anxiety and depression and thus reducing burnout in health care workers and increase the number of satisfied patients in terms of quality of service, right attitude towards them [10,20].

The solution to the problem of emotional burnout should begin with the stage of preparation of future doctors, paramedics to choose a profession, at the scene of training, during the performance of professional duties. According to the authors [3,7], an essential factor in overcoming OER in the early stages is the preparation of students for the future profession of both psychiatrists and psychologists. You need to master the skills of understanding other people, emotional stability, self-regulation, social maturity of the individual, learning the skills of relaxation, passionate culture, and competence. Every healthcare professional needs to be able to cope successfully and consistently with COPD and minimize it. But unfortunately, today requires not only from future doctors, nurses, emotional stability, self-regulation, social maturity, but also psychological culture in society as a whole [12].

Preventive measures to prevent COPD in medical staff also include: conducting psychological training, lectures, learning techniques, and methods of self-regulation of the communicative, emotional, volitional, motivational sphere of personality. The professional activity of doctors and SMP requires constant adaptation to the performance of professional duties without harm to the patient, relatives of the patient, colleagues, and relatives by continually carrying out preventive and corrective measures of emotional state, competence, the culture of health care workers.

In the perspective of further research is the study of an effective system of prevention of PWD in the medical staff of the CHP.

Conclusions. As a result of a study on the detection of emotional and occupational burnout syndromes in the medical staff of the regional psychiatric hospital in Vinnytsia, which was attended by 224 experienced medical professionals with more than ten years of experience, the following has established.

1. Among the studied contingents, women working in the positions of midwives are the most psychologically resilient, and the degree of formation of "Exhaustion" in the last phase of the PREV is the lowest (11.1%). SMP men, on the other hand, are the most vulnerable to OER and the formation rates in each phase of OER are the highest in comparison with other studied contingents and increasing in the dynamics of OER formation and were: in the stress phase – 22.6%, phase "Resistance" – 35.5%, in the aspect of "Depletion" – 39.1%. Given that the IEC, according to ICD-11 (2019), is classified as a pre-disease manifestation (rubrication: "Adaptation disorders" – F43), every second or third male SMP specialist needs psychological help for emotional burnout.

2. Medical staff with higher education by gender had no differences. Still, the indicators of formation in each phase of the EWC were significantly high: in the stress phase in male doctors – 19.4%, in women-doctors – 20.0%; in the "Resistance" phase – 35.5% and 36.0%, respectively; in the stage of "Depletion" – 11.3% and 12.0%, respectively. Therefore, every tenth doctor needs psychological rehabilitation for PREV.

3. When comparing the degree of formation of PER in specialists with secondary and specialists with higher education, it has found that the phase of the stress of psycho-emotional state in medical workers was associated with actual experiences: traumatic circumstances doctors – 14.2 points, SMP – 14.1 balls; signs of anxiety and depressive response doctors – 10.6 points, SMP – 9.9 points; dissatisfaction with themselves doctors – 9.5 points, SMP – 9.2 points, which did not reveal a significant difference between the specified medical staff. However, the feeling of being driven into a cell in the SMP has registered at the level of 7.5 points, which was 1.3 times (5.5 points) higher than in physicians at $p<0.02$.

4. In the formation of the phase of emotional exhaustion of P.V. syndrome, the leading symptoms of psychosomatic and psychophysiological disorders have probably more pronounced in SMP – 10.4 points than in doctors – 8.4 points (at $p<0.05$). A high level of emotional exhaustion has observed in 33.3% of men SMP, which was 1.7 times higher than women (women-doctors – 19.4%, women SMP – 19.5%) and 2.7 times higher than men-doctors (12.0%). A very high rate of emotional exhaustion was observed only in women. In SMP, this figure (14.8%) was 4.6 times higher than in doctors (3.2%) due to their higher emotional lability and rapid emotional exhaustion.

5. Depersonalization (cynicism) has observed in 69.5% of women with SPM; in 66% of women doctors, in men-doctors and men of SMP, these indicators were registered at the level of 40% and 55.5%, respectively. These results are indicating a decrease in empathy and cynical treatment of patients by 2/3 of female medical staff and every other male medical team and create a stigma for the mentally ill, which is unacceptable.

6. Reduction of personal achievements, in other words, feelings of incompetence in their professional field, was observed in 57.9% of women SMP, 46.7% of women-doctors, 42% of men-doctors, and 33.3% of men SMP.

These studies indicate the presence of harmful occupational conditions that lead to high levels of emotional exhaustion, depersonalization, reduction of professional achievements, and calls into question the possibility of continuing to perform their professional duties by every second health worker and require outpatient treatment to prevent the transition of syndromes. PREV and P.V. in diseases.

To solve the problem of burnout in health care workers who works in psychiatric hospitals and prevent their transition to disease, the following measures have proposed:

1. Improving the material and technical base of mental health care facilities and creating the best conditions for the organization of health and safety conditions for health workers, in particular, the arrangement of psychological relief rooms for health workers in each department.

2. It has recommended conducting pieces of training, lectures, conferences on psychological and psychiatric topics with elements of medical ethics, morality, and deontology among doctors and SMP and parts of training aimed at developing stress resistance in medical staff of psychiatric wards – 2 times a year based on the principal workplace.

3. To identify the initial stages of the formation of occupational stress in psychiatric wards, it has recommended conducting psychodiagnostic screening 1-2 times a year.

4. It is expedient to include a set of classes, the training aimed at informing them about the mechanisms of formation and clinical characteristics of occupational stress, as well as the development of resistance to occupational stress in the system of pre- and postgraduate education of doctors and secondary medical workers of psychiatric departments.

5. It has recommended developing a network of individual psychological counseling of medical workers of psychiatric wards of the CHC at the principal place of work, experiencing occupational stress, reduced professional efficiency, and timely detection of health disorders.

6. It is advisable to introduce the diagnosis of somatoform disorders to prevent somatic pathology during the mandatory medical examination with testing by psychologists - once a year.

All of the above measures will help prevent burnout in health

care workers in psychiatric hospitals, prevent its transition to disease, and destigmatize patients.

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SUMMARY

BURNOUT IN MENTAL HEALTH PROFESSIONALS AND THE MEASURES TO PREVENT IT

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The article presents a retrospective analysis of the concept of occupational and emotional burnout syndrome in medical professionals in the field of mental health. The analysis of domestic and foreign scientific sources, biblio-semantic, analytical, and statistical research methods had used in work. The leading causes of burnout and their factors had identified.

The study involved 224 respondents – the medical staff of the regional psychiatric hospital in Vinnytsia. Among the subjects were women – 84.8%, and men – 15.2%. Of the total number of subjects, doctors accounted for 38.8% (87 people), nurses – 61.2% (137 people). The average age of respondents among doctors was 44.6±12.2 years, among SMP 37.2±11.4 years. Work experience in professional activities was: among doctors – 19.7±12.3 years and SMP – 15.5±11.1 years.

For the experimental study had used, the psychodiagnostic method of emotional exhaustion Boyko V.V. and the adapted method of Vodopyanova N.E., The significance of the difference, was assessed using Student's t-test (t).

Recommendations for mental health prevention measures for mental health professionals have developed. The prospect of further research on the problem of burnout is to study an effective system of prevention for the medical staff of health care institutions in Ukraine.

Keywords: predictors of emotional burnout development, psychiatrists, medical workers, occupational stress, prevention.

РЕЗЮМЕ

ПРОФЕССИОНАЛЬНОЕ ВЫГОРАНИЕ У МЕДИЦИНСКИХ РАБОТНИКОВ СФЕРЫ ОХРАНЫ ПСИХИЧЕСКОГО ЗДОРОВЬЯ И МЕРЫ ПО ЕГО ПРЕДОТВРАЩЕНИЮ

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В статье представлен ретроспективный анализ понятия синдрома профессионального выгорания у медицинских работников сферы охраны психического здоровья. Проведен анализ отечественных и зарубежных научных источников, использованы библиосемантический, аналитический и статистический методы исследования. Определены основные причины и факторы профессионального выгорания.

В иследовании приняли участие 224 респондента – медицинские работники Обласной психиатрической больницы г. Винница. Среди участников женщин было 190 (84,8%), мужчин – 34 (15,2%). Из общего числа участников врачей было 87 (38,8%), средний медицинский персонал (СМП) – 137 (61,2%). Средний возраст респондентов среди врачей составил 44,6±12,2 г., среди СМП – 37,2±11,4 г. Стаж работы врачей по профессиональной деятельности составил 19,7±12,3 г., СМП – 15,5±11,1 г.

В ходе исследования использованы психодиагностический метод эмоционального выгорания Бойко В.В. и адаптированная методика Водопьяновой Н.Е. Достоверность различий оценивали с помощью t-критерия Стьюдента.

На основании анализа полученных результатов разработаны рекомендации по мерам предотвращения выгорания у медицинских работников сферы охраны психического здоровья.

რეზიუმე

პროფესიული გადაწვა ფსიქიკური ჯანმრთელობის დაცვის სფეროს მედიცინის მუშაკებში და ღონისძიებები მისი თავიდან აცილებისათვის

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[1]ვინიცას ნ.პიროგოვის სახ. ეროვნული სამედიცინო უნივერსიტეტი; [2]უკრაინის მედიცინის მეცნიერებათა ეროვნული აკადემიის ა.მარზეევის სახ. საზოგადოებრივი ჯანმრთელობის ინსტიტუტი, კიევი, უკრაინა

სტატიაში წარმოადგენილია პროფესიული გადაწვის ცნების რეტროსპექტული ანალიზი ფსიქიკური ჯანმრთელობის დაცვის სფეროს მედიცინის მუშაკებში. ჩატარებულია სამამულო და უცხოური წყაროების ანალიზი,გამოყენებულია კვლევის ბიბლიოსემანტიკური, ანალიტიკური და სტატისტიკური მეთოდები. განსაზღვრულია პროფესიული გადაწვის სინდრომის ძირითადი მიზეზები და ფაქტორები.

კვლევაში მონაწილეობა მიიღო 224 რესპოდენტმა – ქ. ვინიცას საოლქო ფსიქიატრიული საავადმყოფოს მედიცინის მუშაკები. კვლევაში ჩართულთა შორის 190 (84,8%) იყო ქალი, 34 (15,2%) – მამაკაცი. მონაწილეთა საერთო რაოდენობიდან ექიმი იყო 87 (38,8%), საშუალო სამედიცინო პერსონალი - 137 (61,2%); ექიმ-რესპოდენტების საშუალო ასაკი - 44,6±12,2წ., საშუალო სამედიცინო პერსონალის - 37,2±11,4 წ. ექიმების პროფესიული საქმიანობის სტაჟმა შეადგინა 19,7±12,3 წ., საშუალო სამედიცინო პერსონალის - 15,5±11,1 წ.

კვლევის პროცესში გამოყენებულია ემოციური გადაწვის განსაზღვრის ვ. ბოიკოს ფსიქოდიაგნოსტიკური მეთოდი და ნ. ვოდოპიანოვას ადაპტირებული მეთოდი. განსხვავებათა სარწმუნობა ფასდებოდა სტიუდენტის t-კრიტერიუმის გამოყენებით.

მიღებული შედეგების ანალიზის საფუძველზე შემუშავებულია რეკომენდაციები ღონისძიებებთან დაკავშირებით პროფესიული გადაწვის თავიდან აცილებისათვის ფსიქიკური ჯანმრთელობის დაცვის სფეროს მედიცინის მუშაკებში.

THE PECULIARITY OF COVID- 19 GENOME AND THE CORONAVIRUS RNA TRANSLATION PROCESS AS APOTENTIAL TARGET FOR ETIOTROPIC MEDICATIONSWITH ADENINE AND OTHER NUCLEOTIDE ANALOGUES (REVIEW)

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Coronaviruses (CoVs) belong to the family Coronaviridae. They are further subdivided into four genera: alphacoronavirus (α-CoV), betacoronavirus (β-CoV), gammacoronavirus (γ-CoV) and deltacoronavirus (δ-CoV)[6,48]. Both α- and β-CoVs infect mammals, while δ- and γ-CoVs infect birds. In early December 2019, the cases of pneumonia of unknown etiology were reported in Wuhan. After identifying the genome sequence of infected patients, it was revealed that the causative agent was a new type of coronavirus, namely SARS-CoV-2. Like SARS-CoV and MERS-CoV, the newly formed SARS-CoV-2 virus belongs to the group of β-CoVs.

The incubation period for SARS-CoV-2 is on average 3-7 days, although it can be up to 2-14 days [28, 43], which coincides with other known CoV incubation periods (e.g., SARS-CoV incubation period is on average 5 days, although it may increase to 2–14 days [4], the incubation period for MERS-CoV is approximately 5-7 days, although it can be up to 2-14 das [5, 34]. Asymptomatic patients can effectively transmit SARS-CoV-2 during the incubation period, [24].

The goal of the present article is to summarize and analysis the literature data,concerning specific features of COVID -19 virus and to consider the potential targets for etiotropic therapy.

Genetic sequence. The genetic sequence of SARS-CoV-2 is 70% similar to the SARS-CoV sequence. Like the latter, SARS-CoV-2 uses the ACE2 (Angiotensin-Converting Enzyme/Enzyme 2) receptor to enter the cell and infect humans [46,54]. However, with the main antigen component, i.e. the S protein, it differs significantly from its predecessor. The S protein of SARS-CoV-2 binds to the human ACE2 receptor at 10- to 20-fold higher affinity, facilitating the spread of the virus among humans [44]. It should also be noted that the respiratory tracts are not the only route of transmission of the SARS-CoV-2 virus, and it is also transmitted even during close contact. Recent studies have shown that some patients with confirmed COVID 19 experience dyspeptic symptoms such as diarrhea, vomiting, nausea [22,32]. The enteric symptoms of COVID 19 are associated with the presence of the ACE2 receptors in the digestive tract [37].

SARS-CoV-2 uses the genomic RNA as a template to translate pp1a (polyprotein 1a) and pp1ab (polyprotein 1ab). These proteins (pp1a, pp1ab) produce nonstructural proteins (MSPs) in double-membrane vesicles (DMVs) to form the replication/transcription complex (RTC) [25].

Negative-stranded RNA (Coronavirus Genomic RNA (-)) is produced by replication of the complex. As a result of the transient transcription of its RNA-dependent RNA polymerase (RdRp), subgenomic RNAs of different lengths (sgRNAs) are produced [9]. Translation of each sgRNAs results in the production of viral proteins, and replication of negative-stranded RNA (Coronavirus Genomic RNA (-)) yields positive-stranded RNA (+).

The SARS-CoV-2 genome and subgenome contain at least 6 open reading frames (ORFs). The first ORF (ORF1a/b) is 2/3 of the total genome length of SARS-CoV-2; it produces two polypeptides pp1a and pp1ab. Proteolytic cleavage of ORF1a/b results in producing 15/16 NSPs (nonstructural proteins), 4 structural proteins, and 5 complimentary proteins (ORF3a, ORF6, ORF7a, ORF8, and ORF9) [23,47].

ORF1a encodes the production of pp1a, a molecular weight of which is 486 kDa. The pp1a protein contains Plpro (the papain-like protease), 3CLpro, and two membrane proteins MP1 (nsp4 - nonstructural protein 4) and MP2 (nsp6 - nonstructural protein 6).

NSP1 inhibits the synthesis of the cellular proteins in an infected cell. The cell is forced to regulate mainly viral protein synthesis. Moreover, the protein NSP1 does not allow the cellular antiviral proteins to aggregate that is necessary to stop the virus [17,40]. The function of the protein NSP2 has not been determined, only its ability to participate in the placement of endosomes along the cell has been identified [16,29]. The protein NSP3 performs two important functions: 1 -it provides the release of other viral proteins after which they begin to perform their own function; 2 - it changes the function of the proteins of the infected cell.

NSP3 is released by pp1a/1ab via a papain-like protease domain that is part of NSP3 itself [7].NSP4, along with other proteins, is involved in the formation of fluid-containing blisters in an infected cell[38]. NSP5 is specialized in breaking down proteins, causing other NSPs to be activated and start to act [3,55,56].NSP6 is involved in the formation of viral blisters along with the NSP3 and the NSP4 proteins [12,27]. NSP7 and NSP8 help NSP12 generate a copy of the virus RNA genome that gives rise to offspring viruses [14]. NSP9 penetrates into the nucleus with the help of small channels in the cell nucleus and influences the movement of molecules from the nucleus [13,51].The protein NSP10, along with NSP16, disguises a viral gene and prevents the attack of antiviral proteins in human cells that have the ability to detect and destroy viral RNA [8,15]. The function of the NSP11 protein is unknown [2]. The Protein NSP11, together with NSP12,concentrates nucleotides in the coronavirus genome. The ability of the antiviral drug remdesivir to interact with the coronavirus NSP12protein has been identified; studies are being carried out regarding the widespread use of this drug in treatment [1,41].

In a normal state, the viral RNA is twisted. It is assumed that the NSP13 protein destroys viral RNA and thus makes it available for action on proteins involved in the production of new viral copies [11]. The NSP14 protein correctsthe errors (incorrectly added nucleotides) made by the NSP12 protein during duplication of the coronavirus [15,52]. The protein NSP15 [53] supposedlyprotects the virus from the antiviral activities of the cell [19].

The underlying ORF1b is expressed as a pp1a fusion protein through a mechanism that involves the movement of the ribosomal backbone during translation [20,26].The result is the protein pp1ab (≈790 kDa) that already contains ORF 1b containing the helicase domain (nsp13) [39], exonuclease(nsp14), endoribonuclease (nsp15), and nsp16.

The remaining ORFs make up about one-third of the genome length, are located near the 3'-end, and encode at least four types of structural proteins:

- The E protein - a structural protein of the coronavirus membrane that forms the lipid vesicles of the virus. Inside the cell, these vesicles fix proteins that are involved in the human gene regulation process.

- The M protein - a membrane protein of coronavirus. It participates in the formation of the outer membrane of the virus;

- The S-S protein - forms the protective outer layer of the coronavirus RNA genome on the surface of the virus. In micrographs, the club-shaped spikes that stud the surface of coronaviruses are glycoproteins that give the appearance of a radiate crown. Their parts expand and attach to the ACE2 protein in human airway cells. Then it enters the cell.

- The N protein - protects viral RNA, promotes the internal stability of the virus. Most of the N proteins coalesce into a long helix and lead to the formation of the helical nucleocapsid.

So, the SARS-CoV (COVID-19) genome encodes the so-called "auxiliary proteins" that create a favorable environment inside the cell of the host organism, which promotes its multiplication. The ORF3a protein damages the host cell membrane, thus allowing new viruses to come out of the cell. This is what causes pneumonia – a symptom typical for COVID-19.

ORF6 inhibits the signals sent by the infected cell to the immune system, in addition, it inhibits the activity of some proteins in the cell.

When the virus starts coming out of an infected cell, the cell can bind it with the help of the tetherin protein. ORF7 is thought to reduce the supply of the tetherin protein in an infected cell, making it easier for viruses to leave the cell. It also provokes "cell suicide" (apoptosis) that significantly damages the lungs. The function of the ORF8protein is unknown. ORF9b and ORF9c are coronavirus "auxiliary proteins"; ORF9b inhibits the action of the key proteininterferon in the fight against cellular viruses; the function of the ORF9c protein is unknown.

Treatment. The general treatment strategy for COVID-19 involves bed rest and controlled intake of adjuvant medications. It is also recommended to maintain water and electrolyte balance while monitoring other vital parameters (heart rate, blood pressure, pulse, respiration rate, etc.). Some scientists are counting on the possible antiviral effects of IFNα.

Interferon-alpha (IFNα) belongs to the type I IFN family. It plays an important role in resistance to viral infections, inhibits viral infections by interfering with virus replication, and activates the host's immune response. In vitro experiments have shown that IFNα effectively inhibits SARS-CoV replication[46,47].As revealed, IFNα protects cynomolgus macaques from SARS CoV [18,31,57]. Moreover, pilot clinical trials have shown positive therapeutic effects when using IFNα in patients with SARS [46].

Table lists the medications used to treat COVID-19 and shows the targets for their action.

As shown in Table, all of the antiviral drugs listed above have some antiviral (anti-SARS-CoV2) effects, and may have a certainresult on the process of treating SARS-CoV2. The main targets of current medications are:

- Viral RNA-dependent RNA polymerase RdRp (Remdisivir inhibits RNA-dependent RNA polymerase (RdRp), thus blocking the production of viral proteins; However, in contrast, 3-5 exoribonucleases of the virus inhibit the action of remdesivir and reduce the antiviral effect of this drug);

- Viral 3Clpro or PLpro (the papain-like proteins lopinavir/ritonavir block already formed proteins, thus preventing further production of viral proteins);

- The Virus transmembrane S protein and transmembrane protease serine-2 [TMPRSS2] inhibitors(arbidol and Camostat mesylate] can prevent the interaction of the S protein and the cellular receptor ACE2);

- The ACE 2 receptor on the host cell membrane that provides the entry point for the Sprotein(chloroquine and hydroxychloroquine inhibit endocytosis by increasing endosomal pH). Chloroquine can also inhibit RdRp by increasing intracellular zinc concentrations like remdesivir.

However, several key issues need to be emphasized: (1) The potential interaction of these antiviral drugs with other types of medications should be taken into account; (2) Side effects of two medication lopinavir/ritonavir should be considered(dyspepsia and liver damage); (3) Using three or more «antiviral» drugs with different mechanisms considering the side effects of some of them is controversial.

In addition to the medications listed above, research on the possibility of using antiviral antibodies in the plasma of recovered patientsis being carried out intensively. Plasma therapy is commonly used in viral diseases such as influenza A (H5N1), poliomyelitis, and Ebola [10,50].

Table. List of the medications used to treat COVID-19 and the targets for their action

Therapeutic Target	Function	Potential Medications	References
RNA-dependent RNA polymerase (RdRp)	Coronavirus genome replication	Remdisivir and ribavirin.They have an ability to inhibit RdRp.	[36, 44]
ThePapain-like proteasePLpro	Converts viral polyprotein into a functional enzyme.	Lopinavir, protease inhibitor that can inhibit viral protease: 3CLproorPLpro	[52]
The main protease 3CLpro	Converts viral polyprotein into a functional protein	Lopinavir	[21]
The Sprotein and TMPRSS2	The S protein of on the Virus surface that binds to the host surface ACE2 (angiotensin-converting enzyme/enzyme2) receptor.TMPRSS2 'supplements' the S protein to bind to theACE2 (angiotensin-converting enzyme/enzyme2) receptor.	Arbidol - It can prevent the interaction of the S protein and the ACE2 receptor and inhibit membrane fusion. Camostat mesylate inhibits TMPRSS2.	[33, 35]
ACE 2	A receptor on a host cell providing the entry point for the S-protein.	Chloroquine and hydroxychloroquine inhibit endocytosis byincreasing endosomal pH.	[30,42]

Given the above-mentioned facts, in order to stop the spread of coronavirus infections and to avoid their damaging effects, it is promising to inhibit the production of NSPs of the viral origin, which is possible by replacing nucleotides, in particular adenine, in the viral RNA translation phase. The improved models of adenosine analogs such as remdesivir and NITD008 should be used for this purpose [49, 50] because both of them try to inhibit the viral replication process by inhibiting RdRp. In addition, it is known that 3'-5 exoribonucleases of SARS-CoV2 block the inhibitory effect of remdesivir on RdRp, promoting further replication of the virus. Therefore, it is necessary to create new modified/improved versions of remdisivir and other adenosine analogs.

Conclusion. The cases of SARS-CoV were first reported in 2002 and the virus quickly spread to 32 countries around the world. Ten years later, MERS-CoV became widespread in 2012, and eight years later, in 2020, a new viral infection SARS-CoV-2 emerged. It has been proven that SARS-CoV-2 enters the cell with the help of the ACE2 receptors. The fact that this type of receptors is found not only in the respiratory system but also in the liver tissues, the digestive system (small intestine, duodenum), testicles and kidneys, makes these organs highly vulnerable to SARS-CoV-2.The different group of drugs have been proposed in complex treatment of COVID-19.Despite of this coronavirus is still associated with high incidence of various complications and fatal outcome worldwide .According epidemiologic studies the most susceptible are the patients with accompanying diabetes, cardiovascular ,respiratory system diseases and obesity.Potentially patients with intestinal microbiome disorders also may become vulnerable to COVID -19[32].

Given the global health threat caused by SARS-CoV-2, there is an urgent need for effective prevention and treatment of COVID-19 pneumonia, although finding drugs to treat pathogenic SARS-CoV-2 still remains a major problem. The medicines available to doctors around the world do not have a significant detrimental effect on the virus, as evidenced by the current epidemiological data.In initial stage the introduction of adenosine analog remdesivir against COVID-19 was considered as perspective drug[36].This agent was approved or authorized in about 50 countries, including USA and EU, but currently there are controversial views regarding its ability to reduce mortality in COVID 19.

We suppose that if the improved versions of adenosine analogs (NITD008, Remdesivir, etc.) with more efficacy and safety are developed, the virus will not be able to have a detrimental effect on host cells because they (the improved versions of adenosine analogs) will have the ability to inhibit the virus translation process rather than RdRp. As a result, the virus will no longer be able to produce non-structural proteins (nsps) so important for the manifestation of viral activity.

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SUMMARY

THE PECULIARITY OF COVID- 19 GENOME AND THE CORONAVIRUS RNA TRANSLATION PROCESS AS APOTENTIAL TARGET FOR ETIOTROPIC MEDICATIONSWITH ADENINE AND OTHER NUCLEOTIDE ANALOGUES (REVIEW)

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Despite the multifaceted effects of the medicines provided for COVID-19treatment, the number of the infected and mortality of patients increases which demonstrates the insufficient effectiveness of drugs used to fight coronavirus infections in medical practice, and clearly shows the need to develop new treatment tactics.In this review article are summarized and analyzed the literature data concerning specific features of COVID 19. Particular attention is given to genetic characteristic of this virus ,to mechanism of its invasion into the human organism, replication and interection with ACE-2 receptors ,as well as to the basic targets for the action of existing drugs with antiviral activity against COVID-19.

Currently, the following medications are used to treat COVID-19: remdesivir, chloroquine, hydroxychloroquine (HCQ), ribavirin, lopinavir/ritonavir. According to a recent theory of coronavirus treatment, the starting point for the mechanism of action of a potential etiotropic drug is the inhibition of the coronavirus main protease (Mpro/3CLpro) and the papain-like protease (PLpro). Among the drugs listed above, lopinavir acts through this mechanism but is characterized by severe side effects.It is emphasized that remdesivir as adenosine analog provides inhibitory action on RNA dependent RNA-Polymerase,but there are controversial views about reduction in mortality during using of this drug against COVID-19.

The present paper discusses the mechanism of action of a potential etiotropic drug against coronavirus, which implies the replacement of the nucleotides involved in the process of translation of the virus with their analogs with the aim to “inhibit” the ribosome and block the production of viral proteins.

Keywords:COVID-19, Genetic sequence**,** etiotropic drug, ribosome.

РЕЗЮМЕ

ОСОБЕННОСТИ ГЕНОМА COVID-19 И ТРАНСЛЯЦИОННЫЙ ПРОЦЕСС РНК КОРОНАВИРУСА КАК ПОТЕНЦИАЛЬНАЯ МИШЕНЬ ДЛЯ ЭТИОТРОПНОЙ ТЕРАПИИ АДЕНИНОМ И РАЗНЫМИ АНАЛОГАМИ НУКЛЕОТИДОВ (ОБЗОР)

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Несмотря на многочисленные эффекты лекарственных средств, применяемых для лечения COVID-19, количество инфицированных и смертность пациентов увеличивается, что свидетельствует о недостаточной эффективности препаратов, применяемых в медицинской практике для борьбы с коронавирусными инфекциями, и необходимости разработки новой тактики лечения.

В настоящей обзорной статье суммированы и проанализированы данные литературы, касающиеся специфических черт коронавируса. Особое внимание уделяется генетической характеристике этого вируса, механизму его инвазии в человеческий организм, репликации и взаймодействию с АКФ-2 рецепторами, также как и основным мишеням для действия существующих лекарств, обладающих антивирусной активностью против коронавируса.

В настоящее время для лечения COVID-19 используются следующие препараты: ремдесивир, хлорохин, гидроксихлорохин (HCQ), рибавирин, лопинавир/ритонавир. Согласно существующей теории лечения коронавируса, отправной точкой для механизма действия потенциального этиотропного препарата является ингибирование основной протеазы коронавируса (Mpro/3CLpro) и папаин-подобной протеазы (PLpro). Среди вышеперечисленных препаратов лопинавир действует повредством этого механизма, однако характеризуется серьезными побочными эффектами. В данной статье обсуждается механизм действия потенциального этиотропного препарата против коронавируса, который подразумевает замену нуклеотидов, участвующих в процессе трансляции вируса, их аналогами с целью «ингибировать» рибосомы и блокировать производство вирусных белков.

რეზიუმე

კოვიდ-19-ის გენომის თავისებურებანი და კორონავირუსის რნმ-ის ტრანსლაციური პროცესი, როგორც პოტენციური სამიზნე ადენინით და ნუკლეოტიდების სხვადასხვა ანალოგებით ეტიოტროპული თერაპიისთვის (მიმოხილვა)

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COVID-19-ის სამკურნალოდ მოწოდებული მედიკამენტების მრავალმხრივი ეფექტების მიუხედავად, ინფიცირებულთა რიცხვი და პაციენტთა სიკვდილიანობა მატულობს, რაც ცხადყოფს კორონავირუსის ინფექციებთან ბრძოლის სამკურნალოდ გამოყენებული მედიკამენტების არასაკმარის ეფექტურობას და მკურნალობის ახალი ტაქტიკის შემუშავების აუცილებობას. სტატიაში სუმირებული და გაანალიზებულია ლიტერატურული მონაცემები, რომლებიც ეხება კორონავირუსის სპეციფიკურ ნიშნებს, კერძოდ, განსაკუთრებული ყურადღება გამახვილებულია ამ ვირუსის გენეტიკურ მახასიათებლებზე, ადამიანის ორგანიზმში მისი ინვაზიის, რეპლიკაციის მექანიზმზე და ამფ-2 რეცეპტორებთან ურთიერთქმედებაზე, ისევე როგორც კორონავირუსის საწინააღმდეგოდ მოქმედი ანტივირუსული ეფექტის მქონე არსებული პრეპარატების ძირითად სამიზნეებზე.

ამჟამად COVID-19-ის სამკურნალოდ გამოიყენება შემდეგი მედიკამენტები: რემდესივირი, ქლოროქინი, ჰიდროქსიქლოროკინი (HCQ), რიბავირინი, ლოპინავირი/რიტონავირი. კორონავირუსის მკურნალობის ბოლოდროინდელი თეორიის თანახმად, პოტენციური ეტიოტროპული პრეპარატის მოქმედების მექანიზმის ამოსავალი წერტილი არის კორონავირუსის მთავარი პროტეაზას (Mpro/3CLpro) და პაპაინის მსგავსი პროტეაზას (PLpro) დათრგუნვა. ზემოთ ჩამოთვლილ მედიკამენტებს შორის ლოპინავირი მოქმედებს ამ მექანიზმის საშუალებით, მაგრამ მას ახასიათებს მწვავე გვერდითი მოვლენები.

წინამდებარე ნაშრომში განხილულია კორონავირუსის საწინააღმდეგო პოტენციური ეტიოტროპული პრეპარატის მოქმედების მექანიზმი, რაც გულისხმობს ვირუსის რეპლიკაციის პროცესში ჩართული ნუკლეოტიდების ჩანაცვლებას მათი ანალოგებით რიბოსომის "დათრგუნვის" და ვირუსული ცილების წარმოების ბლოკირების მიზნით.

LIVER EXTRACELLULAR MATRIX PECULIARITIES IN MAMMALS AND AVIANS

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The extracellular matrix - the connective tissue framework of the liver - on the one hand, determines the shape of the organ, and on the other hand, creates specialized compartments for blood and lymphatic vessels and nerves, as well as cell populations, the synergy of which determines the various functioning of the organ. The liver is the largest and heaviest parenchymal organ, and an appropriate matrix design is required to maintain its shape and fix it on the abdominal walls [1]. The liver has a dual blood supply (arterial and portal), and the connective tissue spaces containing these vessels are built with this factor in mind. Unlike other organs, in which there are three circulating fluids and, therefore, there are three compartments for the microcirculation, four fluids circulate in the liver: blood, bile, interstitial juice, and lymph [2]. At the same time, the liver produces more lymph than any other organ (up to 50% of the total amount of lymph in the body). Thus, the liver matrix forms a highly complex but strongly regulated labyrinth in which liver cells, blood vessels, bile ducts, lymphatic ducts, and tissue fluid have their own but closely interconnected compartments [3-5].

The study of the liver connective tissue skeleton dates back to the 17th century. Pursuant to Couinaud [6], in 1640 Walaeus described the connective tissue sheath, which wraps the portal vein, the hepatic artery, the bile duct, the lymphatic duct, and the nerves entering and leaving the liver connects to the capsule of the liver and hepatoduodenal ligament. Walaeus sheath originates from the vasculobiliary sheath (Glisson's capsule) and is not derived from the peritoneum or the capsule of the liver (Laennec's capsule). Besides, the separation between Laennec's capsule and the Walaeus sheath can be seen microscopically at the hepatic hilum [6], where the Walaeus sheath forms a thick plate at the inferior part of the liver referred to as the hilar (portal) plate [7].

The portal pedicle wrapped by the Walaeus sheath continues inside the organ, as the so-called Glissonian Pedicals [8].

Until the late 1980s, it was thought that the branches of the portal vein and the hepatic veins in the liver were mutually autonomous and that their connective tissue sheaths did not touch each other [9-11]. There is the established opinion in the modern textbooks of Hepatology that Glisson's portal pedicles and the main branches of the hepatic veins spatially intersect, but the parenchymal area remains between them and, therefore, they are anatomically independent of each other [12]. But Ilia Chanukvadze showed that in the human liver the connective tissues of the main portal complexes and hepatic veins might merge in some zones of the intersection. The regions of such merging were called Portacaval Fibrous Connections (PCFC). Besides, the various forms of PCFC - as are the complete merging, touching merging, septal- and fibril-like connections - were described [13,14]. It has been revealed that PCFC, as an anatomical structure, is formed on the 11th-12th weeks of pregnancy [15].

A new wave of research on the connective tissue structures of the liver has been observed over the past five years. This "revisiting" is based on the introduction of new methods and computer technology in morphological studies [16]. This is due, on the one hand, to the creation of the possibility of endoscopic anatomical liver resections and, as a consequence, to the need to clarify the intercommunications of the connective tissue structures of the liver [17], and on the other hand, to the prospects of using human and animal liver matrices as the scaffolds for the creation of bioartificial livers (in turn related to the development of stem cells technology and bioengineering) [18-21].

At the same time, the analysis of these studies makes it obvious that the knowledge on the connective tissue skeleton of the liver lacks systematization, the terminology is inconsistent, and sometimes the construction of this or that component of the liver matrix is addressed controversially in the literature [22]. All of the abovementioned confirms the necessity of the further study of the liver matrix and complex analysis of the results obtained by different methods.

We set a goal to study peculiarities of the construction of connective tissue matrices of the livers of different mammals and birds for the identification and systematization of the general and specific regularities of this structure.

Material and methods. We have studied the relation of the connective tissue sheaths, covering the portal complexes and hepatic veins to each other and to the liver capsule and intralobular connective tissue network – in the livers of mammals with a gallbladder (pigs, sheep) and with no gallbladder (rats) and birds (domestic hens with gallbladder). The livers of the named animals and birds were studied by the anatomical preparation, histological, histotopographical, histochemical, immunohistochemical methods, scanning electron microscopy (SEM) of corrosion casts, and fluorescence microscopy. The number, age, and distribution of studied subjects according to the research methods are shown in Table 1.

Histology. Liver tissue sections of 3-5 μm were stained by the standard H&E method and studied microscopically with different magnification.

Histochemistry. Liver tissue sections of 3-5 μm were stained using Masson's Trichrome kit (Sigma Aldrich Catalog Number: C970D37) according to the recommendation of the manufacturer.

Immunohistochemistry. Liver sections of 3-5 um thickness obtained from the parragin-embedded blocks were stained with pan-Cytokeratin antibody [AE1+AE3] (neat) (ab961, Abcam, plc, Cambridge,UK) using appropriate protocols provided by antibody suppliers. Sections were counterstained with haematoxilin.

Histotopography. For Histotopographic examinations, histological samples of the liver of experimental animals and birds with a thickness of 3-5 micrometers were prepared.

Area of a tissue sample is up to 2cm x 4 cm. Samples were colored with Hematoxylin and Eosin (H&E) and Masson's trichrome stains. Tissue samples were digitized with MoticEasyScan Pro 6-FS scanner with x40 magnification (0.26 μm/pixel resolution). As a result, the original tissue sample was increased 1 000 times. High resolution digital images were visualized by Motic DSAssistant software, in which different types of distance measurement and morphometry tools were used for the analysis of slide images.

Corrosion Casting. The corrosion casts of the portal and hepatic veins of the pigs were prepared by injecting the solidifying liquid latex "Nairit" (Yerevan, Armenia). Latex was injected by the 20-gram graduated standard syringe under the manual pressure, through the catheter fixed a) into the extrahepatic part of the portal vein and b) into the inferior vena cava. The corrosion of liver tissue was performed in 40% sulfuric acid, during 3 days with the following washing in running water. The obtained casts were studied macro- and microscopically by using a light stereomicroscope (ProScope HR device, Bodeline Technologies, US).

The corrosion casts of the portal and hepatic veins of the rats were prepared by injecting the "Protacryl-M" (see below). The abdominal cavity of the Wistar rat, weighing 200-220 g was opened under general ether anesthesia. The catheters with appropriate diameters were inserted in the portal vein and caudal vena cava and fixed with ligatures. The liver vessels were washed out via portal vein catheter with the cocktail including 100 ml 0.9% NaCl, 1,0 ml Atropine, 1,0 ml No-Spa, 1ml Heparin, and 1 ml 2% Novocain. Outflow was achieved through the catheter inserted in the caudal vena cava. Cra-

Table 1. The number, age, and distribution of animals according the research methods

Research method / Mammal/bird	Anatomical preparation	Histologiy, histotopography	Histochemistry	Immunohistochemistry	Fluorescence microscopy	Corrosive specimen	SEM Corrosive specimen	Total
Pig (1 year old)	2	2	2	-	2	2	-	4
Sheep (1 year old)	2	2	2	-	2	-	-	3
Rat (6 months old)	2	6	6	2	2	2	2	14
Hen (6 months old)	3	3	3	-	2	-	-	3

note: Several research methods were used on the same livers

nial vena cava was ligated preliminary. After washing out the liver, the injection of the"Protacryl-M" cocktail ("Protacryl-M" powder 3 cm3 dissolved in 7.5 ml of its liquid component) was performed through both catheters under manual pressure. Cocktail injected in portal vein was colored by red pigment, while the cocktail injected in hepatic veins – by the blue pigment (Protacril powder, liquid component and the pigments are the components of standard "Protacryl-M" kits – Kharkiv, Ukraine). The liver tissue was corroded in 20% KOH solution, in-room temperature after Protacril cocktail polymerization. After 2 hr fragments were washed out in distilled water for 10 min three times, the process was repeated 6 times. Dried casts were studied with the ProScope HR device.

Fluorescent Microscopy. Immunofluorescence images were were obtained by the imunofluorescence microscope Nikon H550L (Japan). The digital images were captured by Infinity 2 camera provided with Infinity software version 6.5.6 – for measuring and analyzing.

Results and discussion. In all investigated livers, around the portal and hepatic veins, including their thinnest branches/tributaries, there are connective tissue sheaths of various thicknesses, consisting of different ratios of different types of connective tissue fibers. These sheaths communicate with each other, as well as with the liver capsule and the interlobular mesh of connective tissue, creating a single extracellular matrix - the connective tissue skeleton of the organ.

Porcine. In the porcine liver, both the portal and caval ports are located close to each other at the dorsal (posterior) surface of the liver (Fig 1a). Large-caliber portal and hepatic veins are arranged on the planes situated more or less parallel to each other. The above-mentioned blood vessels intersect with each other, but only within the mentioned planes. At the same time, their thin branches intersection might happen with different angles (Fig 1b). The hepatic veins are located above the portal vein branches (cranially).

In porcine liver, the mesh-like structure of the connective tissue fibers links the portal tracts with each other and separates the liver lobules of different sizes (from 0.5 mm to 2,2 mm in diameter) and shapes (irregular polygons). The connective tissue septs positioned among the liver lobules are quite thick (5-15 mm). In addition, these septs in some areas involve the connective tissue sheaths of the hepatic vein tributaries. Due to this feature, not rare the connective tissue framework enveloping the classic lobules includes not only the portal tracts but also the tributaries of the hepatic veins, which makes the architecture of the soft skeleton of the liver even more complex. Thus, the connective tissue sheaths of the portal tracts and the hepatic vein tributaries are connected to each other by numerous connective tissue septs separating the liver lobes. The numbers of liver lobules situated between the hepatic veins and the portal tracts might vary from one to several (Fig 1c,d,g,k).

The described fibers of the connective tissue structure continue into the interlobular connective tissue, located in the space of Disse, which proves the formation of a single connective tissue labyrinth, likewise, it was described in cat liver [3] (Fig 1e,f). The liver capsule (the Laennec capsule), consisting of thin elastic and I-type collagen fibers, is connected with the above-mentioned connective tissue interlobular septs, which fibers extend into the intralobular extracellular matrix (Fig 1h). At the same time, in some areas, between the thin connective tissue plates (0.2-0.5 μm) covering liver lobules, from the one hand, and the Glisson's capsules of the large portal pedicles, from the other hand, were found the fissures, which were crossed over by the single connective tissue fibers establishing the communication between the Glisson's plates and the derivates of Laennec's capsule (Fig 1i,j). These plates are similar to those described in the human liver 23,24]. However, we couldn't find the "proper liver ligament" identified by Ikeda et al., between the Laennec's and Glisson's plates using the computer program processing of liver histotopogramms [16]. We think that such identification of "new structure" is somewhat artificial which is resulted from the "ability" of the used computer program.

The described fissure, as well as the connective tissue fissures inside of the Gleason capsule, according to our previous studies, represents one of the compartments of the pre-lymphatic circulation basin [5].

Masson's trichrome differently dyes different connective tissue fibers and muscles (the elastic fibers in pomegranate color, I-type collagen – in blue (flesh-color), and the III type collagen – in brown colors). Besides these structures are characterized by different self-fluorescence lightening in fluorescent microscopy. Considering the above mentioned we can conclude that the connective tissue structures of the liver capsule, interlobular septa, and hepatic vein tributaries sheaths are mainly represented by the interweaving of the I-type collagen and elastic fibers. hepatic vein tributaries sheaths additionally contain the longitudinal smooth muscle fibers, the individual bundles of which are separated from each other by thin connective tissue inserts (Fig 1k,l).

The single smooth muscle fibers are found in portal vein adventitia as well. In addition, the portal vein has its own connective tissue coat enveloping its adventitia. A narrow fissure (gap) can be detected between the adventitia and the mentioned coat. The arteries, bile ducts, and nerve trunks (vagus branches) in the portal tracts are characterized by similar covers (Fig 1m,n). These findings fully agree with the data obtained in the 60s and 70s of the last century by several researchers and summarized by Kovanov and Anikina [25] identifying the fissures around the tubular structures of portal tracts. These fissures represent the areas for the interstitial fluid and pre-lymph circulation and confirm that the extracellular matrix of the liver represents a single basin for the extravascular microcirculation [5].

The walls of the portal vein and bile duct contain elastic fibers. In the walls of bile ducts, these fibers are accompanied by single muscular bundles. Elastic fibers are abundantly represented also in the perineurium, as well as in the subendothelial and external layers of the branches of the hepatic artery.

A significant portion of the connective tissue skeleton of the portal tract is represented by thick, well-structured fibers of the I-type of collagen (Fig 1i,m).

In the porcine liver, we couldn't identify some connective tissue structures described in the human portal tract. In particular, the human portal tract contains 2 connective tissue layers located around the portal vein, the 1st of which, located close to the portal vein, contains the branches of the hepatic arteries, and the other, located peripherally - contains the bile ducts and extramural peribiliary glands. There are no similar layers in the portal tracts of the porcine liver.

The intramural biliary (mucosal) glands are quite common in the bile ducts of the porcine, while the extramural peribilliary glands are rare. Besides, it is notable, that these glands are opened into the biliary lumen from all sides, more or less evenly (Fig 1o), while in humans these glands are usually located along the two opposite sides of the ducts [13,26,27].

Porta-caval connective tissue connections by the merging of the elements of the connective tissue sheaths of large portal tracts and the hepatic veins, we could not find in pig livers. In addition, with some assumptions, the fibril-like or septal connections of the portal and venous sheaths, consisting of connective tissue fibers, can be considered as analogs of Porta-caval connections described in humans (Fig 1c,d,g).

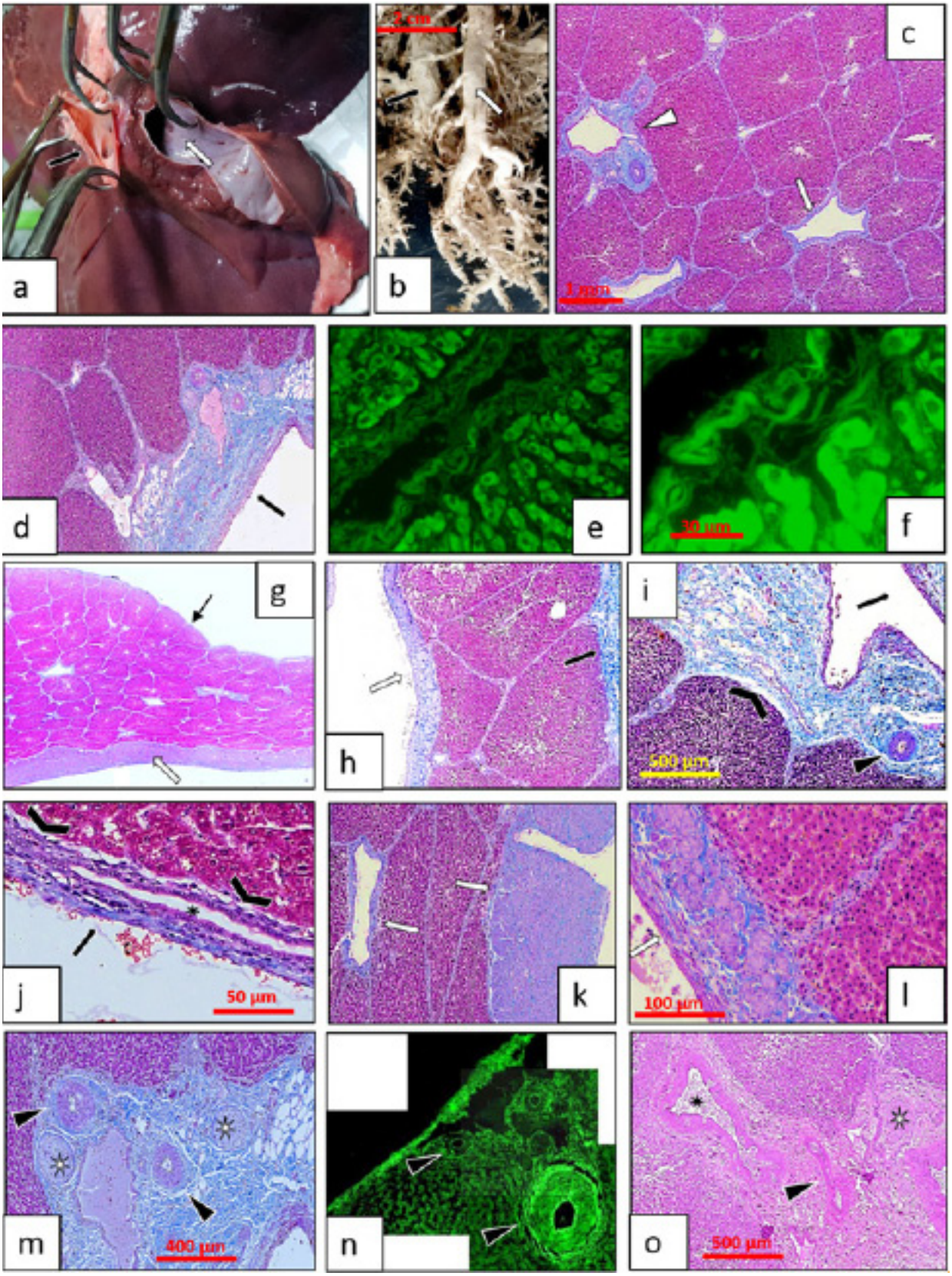

Fig. 1. Porcine Liver. a - the macro-anatomical specimen; b - corrosion casts of portal and hepatic vein; c,d,g,h,i,j,k,l,m – histotopograms of liver tissue (Masson's trichrome staining); e,f,n – fluorescent microscopy of liver tissue (self-fluorescence); o – histology of liver tissue (H&E). White arrow – hepatic veins; black arrow – portal vein; white arrowhead – portal tract; free arrow – hepatic capsule (Laennec's capsule); black arrowhead – hepatic artery; pterygoid arrowhead – fissure; white asterisk – nerve (the branch of vagus); black asterisk – bile duct (bile ductule)

Hen. The portal and caval ports of the hen liver are separated from each other to some extent. The hepatic veins have an extrahepatic section of considerable length (not found in mammals) and the hen liver is connected to the dorsally located inferior (caudal) vena cava by 3-5 hepatic veins covered with peritoneum (Fig 2a). Posteriorly, liver is connected to the small intestine by the ligament, which is created from the duplication of the peritoneum as well, and in the depth of which the bile duct, hepatic portal vein, hepatic artery and the gallbladder are located [28].

Intrahepatic sections of portal complex and hepatic veins are usually located on planes at different angles to each other and therefore spatially intersect with each other.

The structures made of different types of connective tissue fibers are more sharply differentiated within the hen portal tracts in comparison with the porcine liver. In particular, the own capsule (sheath) of the portal vein is formed by the type-I collagen and circular elastic fibers, which are tightly twisted with the portal vein adventitia. The thickness of this capsule does not exceed 20-30 μm. The portal vein with its connective tissue sheath is situated in the connective tissue of the portal tract, which is mainly composed of type-I collagen fibers of different sizes and directions (Fig 2b,c,d,e,f). Sometimes, in the immediate vicinity of the portal vein, they form small areas (spaces) in which longitudinal smooth muscle fibers are located (Fig 2c,g).

In those regions where thin daughter branches separate off the portal vein, the amount of type -I collagen gradually decreases, rarely "disappears momentarily" and the thin branch of the portal vein remains surrounded by a thin sheath (3-5 μm) of type-III collagen and elastic fibers (Fig 2b,c). The fissures between the sheath and adventitia of the portal vein, similar to those found in pig liver might be also found in the hen liver but less frequently (Fig 2g).

The arteries located in the portal tract are characterized by a well-defined muscular layer. At the same time, their adventitia is so thin that it is often impossible to identify. The connective tissue covering around the artery, as it was described in the human and porcine livers, is practically absent in hens. The study of the histotopograms gives the impression that the arteries with a well-developed muscular layer are directly "inserted" into the connective tissue structure of the portal tract, mainly formed by type-I collagen fibers. At the same time, the contact between the

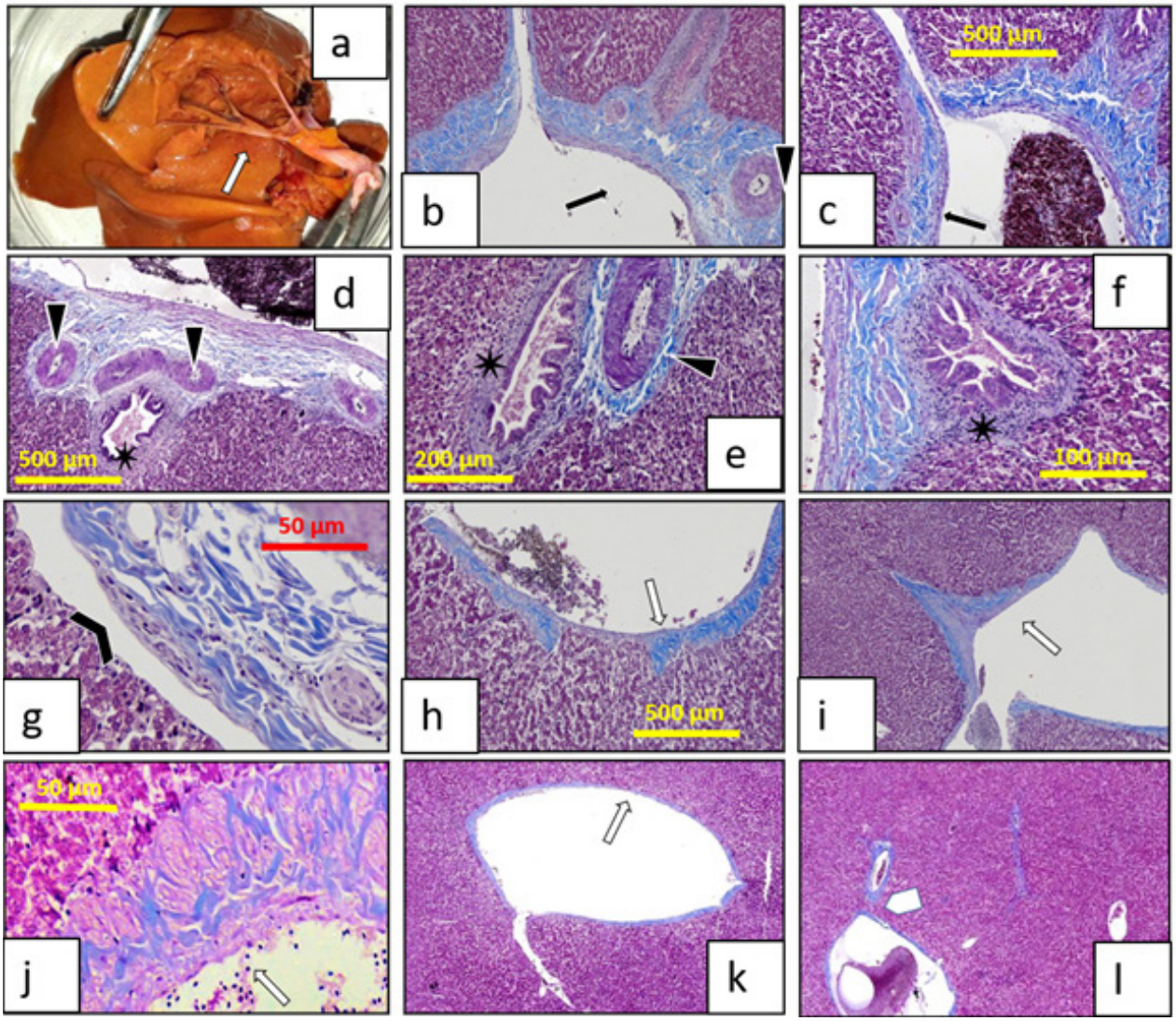

Fig. 2. Hen Liver. a - the macro-anatomical specimen; b - l – histotopograms of liver tissue (Masson's trichrome staining). White arrow – hepatic veins; black arrow – portal vein; white arrowhead – portal tract; free arrow – hepatic capsule (Laennec's capsule); black arrowhead – hepatic artery; pterygoid arrowhead – fissure; white asterisk – nerve (the branch of vagus); black asterisk – bile duct (bile ductule); pentagonal arrow – fibril-like porta-caval fibrous connection

artery wall and surrounding collagen fibers is loose, sometimes to such an extent that it becomes possible to identify thin (1-5 microns) gaps (Fig 2b,d,e).

The wall of the bile ducts is thick. They are covered with a dense peribiliary capsule of type-I collagen fibers, which contains a large number of fibroblasts. The epithelial layer is wrinkled to such an extent, that the lumen of the bile ducts is often star-shaped on the slices. Furthermore, small-diameter ductular profiles lined with epitheliocytes were found in the peribiliary connective tissue around the lumens of bile ducts (Fig 2d,e,f,). This indicates that the hen bile ducts are also supplied by the peribiliary mucous glands. Considering our previous studies it was expected [29] as the hens have gallbladder. The high degree of wrinkling of the epithelial lining of the bile ducts co-exists with the multi-rowed positioning of epithelial cells, which imitates the proliferation.

The bile ducts are always located at the periphery of the portal tract. Its connective tissue sheath is connected to the connective tissue skeleton of the portal tract constituted by type-I collagen fiber from one side, while from the other side directly adjoin to the liver parenchyma. In such regions, the connective tissue fibers of the duct membrane continue directly into the adjacent lobules, thus creating a unified structure of the extracellular matrix of the liver (Fig 2e,f). But in regions where instead of the bile ducts the connective tissue of the portal tract is adjoined to the liver parenchyma, it is possible to identify fissures between these structures. Such fissures are bordered on the one hand by the fibers of the portal tract and on the other, by the capsule directly covering the parenchyma, the separate fibers of which extend into the intralobular matrix (Fig 2g).

The connective tissue fibers, surrounding the large hepatic veins (both type-I and type-III collagen and elastic fibers), are tightly intertwined and form the sheaths of these veins. Besides, the greater the venous lumen the greater the thickness of the connective tissue sheaths (Fig 2h,i). The thickness of the connective tissue sheath around the main veins of the liver reaches 100 μm. The type-I collagen fibers pass through the surrounding layer of the III type collagen and elastic fibers, thus giving the impression of making septums in the mentioned layer. Longitudinal muscle fibers (running alongside the veins) are located in some spaces bordered by these septums (Fig 2 h,j). The thickness of the connective tissue sheath containing such elastic and muscular fibers increases locally at the junctions of the tributaries with the hepatic veins (Fig 2 I,k). It should has a certain sphincter-like and blood-flow-supporting function. The connective tissue sheaths formed around the smaller veins are much thinner and mainly contain type-III collagen fibers, part of which extends directly into the connective tissue skeleton of adjacent lobules.

Generally, PCFC is not observed in chicken liver. The interconnection of portal tracts and separate connective tissue fibers, covering the hepatic veins, is observed only occasionally. Considering their shape, these connections can be called fibrillar-like, or at most, plate-shaped PCFC (Fig 2l).

Rat. The portal and caval ports are located close to each other at the dorsal (posterior) surface of the rat liver (similar to those described in the porcines). The hepatic veins are located above (cranially) the portal veins. The large portal and hepatic veins are located in planes more or less parallel to each other. They intersect with each other, but within the specified plane. In addition, thin branches of the portal vein and hepatic veins can intersect at different angles (Fig 3a).

In the liver of rats, in at least one or two lobes, we found an area where the fibers of the portal connective tissue fuse with the connective tissue surrounding the hepatic vein. Sometimes this fusion might be so intim that above-mentioned two types of veins can have one single (united) wall. Such areas, which are considered to be PCFCs, similar to human PCFCs, are the areas where not only connective tissue fibers can intertwine, but some structures of the portal complexes can be displaced into the connective tissue sheath covering the hepatic veins (Fig 3b,c,d,e,f,g).

The adjoining of the branches of the hepatic artery to the walls of the hepatic veins in the areas of close contact of the portal tracts with the branches of the hepatic veins was described many years ago [30]. More recently, we also found the bile ducts dislocated from the portal tract towards the hepatic veins. Notably, in 2014, we confirmed that translocated ducts can accompany the hepatic veins up to their small tributaries, including the sublobular and central veins; Therefore, we assumed that in some parts of the rat liver, the outflow of bile from the bile capillaries can occur not only in the interlobular bile ducts located in the portal tract but also in the bile ducts passing along the central and/or sub-lobular venules [31].

Various forms of portocaval connections have been identified in rat liver, starting from the complete merging and ending with fibrill-like connections. In the regions of complete fusion, the branch of the hepatic vein is connected with the bilio-vascular triad of the portal complex as the fourth element. This biliary-vascular quaternion are enclosed in a single common capsule - the sheath, similarely to the one described in the human livers [32].

In rat liver, the peculiarities of the location of connective tissue fibers, going along the portal tracts of different sizes and the tributaries of the hepatic veins, substantially repeat those described in the hen liver. In addition, it should be noted that in the liver of rats elastic fibers are found in smaller amounts, as well as the muscular layer of arteries is less vividly pronounced. It is also noteworthy that in the rat liver hilum and in the area of large vessels, the boundary between the Glisson's capsule and the Laennec's capsule is distinguished. The latter, as in the porcine liver, covers not only the liver surface but also separates its parenchyma from the adjoint portal tracts and tributaries of the hepatic veins (Fig 3h,i). Besides, we have confirmed the opinion of other researchers, that the fissures, between the sheats of small portal tracts and hepatic veins, on the one hand, and the connective tissue sheaths of the liver parenchyma, on the other hand, is no longer identified. The fibers of all the named connective tissue structures intertwine, forming a single extracellular carcass of the liver (Fig 3j,k). At the same time, gaps are maintained between the adventitia and connective tissue capsule of individual blood vessels, on the one hand, and the above-mentioned capsule and Glisson's sheath covering the entire portal tract, on the another hand. The results of scanning electron microscopy of the corrosion casts of the hepatic blood vessels presented in Fig 3l,m and described and discussed in detail in our previous papers [33], confirm the above-mentioned. In particular, in some of the corrosion casts, a "leak" of the hardening mass introduced into the portal vein was found. Some of these leaks travel around the vessels - in the spaces bounded by the perivascular capsule. These hardened leaks might form the sheaths that enveloping the casts of the blood vessels. Some sheaths contain casts of two blood vessels, but some are empty. In several samples, we have found the patterns where one empty (free from the vessel) sheath was surrounded by another. In addition, a direct continuation of these sheaths into the casts of intralobular connective tissue spaces (perisinusoidal spaces of Disse) was found. This once again confirms the existence of the extracellular matrix of the liver as a single structure.

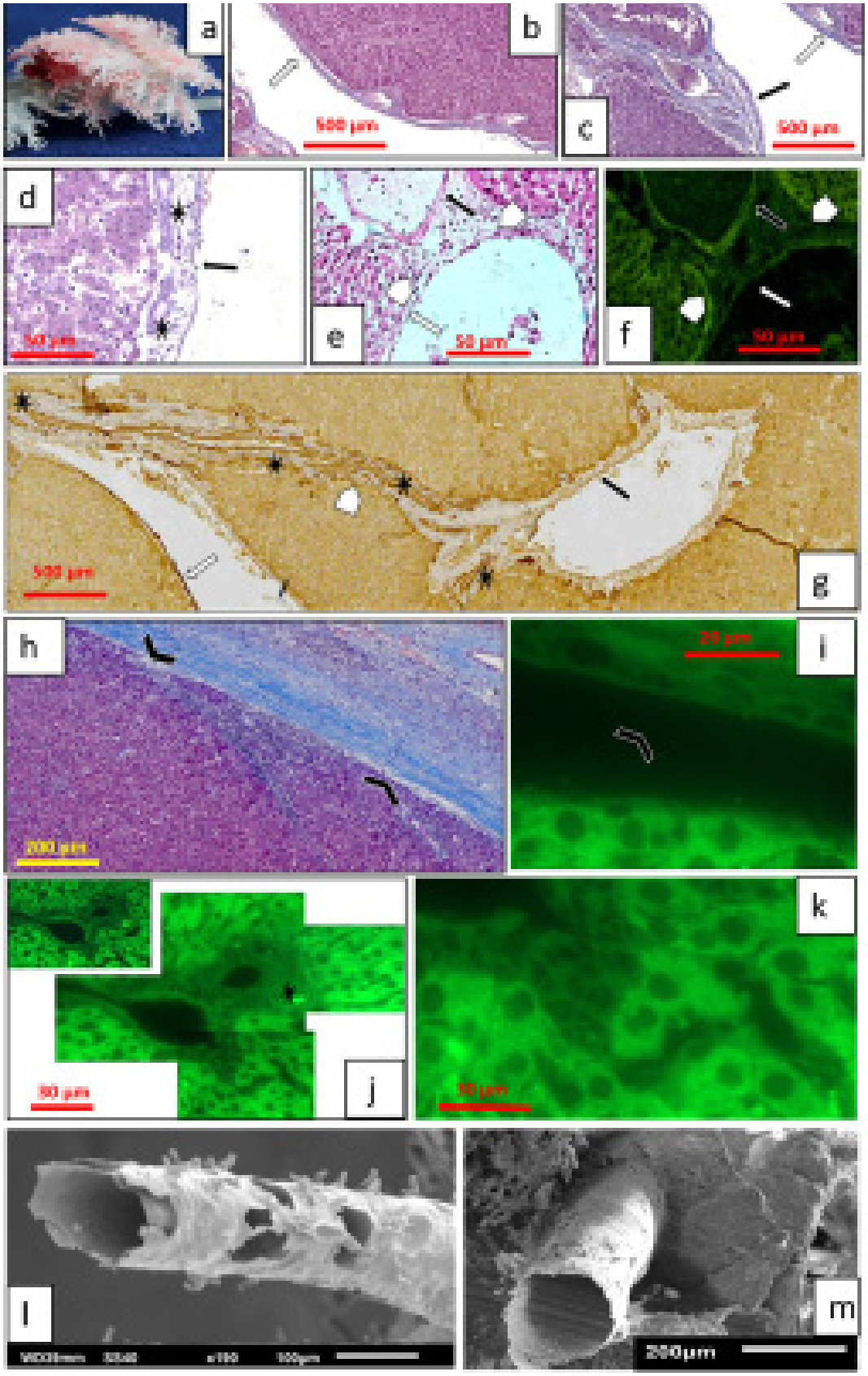

Fig. 3. Rat Liver. a - the corrosion casts of portal (red color) and hepatic (blue) veins; b,c,d,e,h – histotopograms of liver tissue (Masson's trichrome staining); f,i,j,k - fluorescent microscopy of liver tissue (self-fluorescence); g- immunohistochemistry (AE1-AE3) of liver tissue; l,m - scanning electron microscopy of corrosion casts. White arrow – hepatic veins; black arrow – portal vein; pterygoid arrowhead – fissure; black asterisk – bile duct (bile ductule)

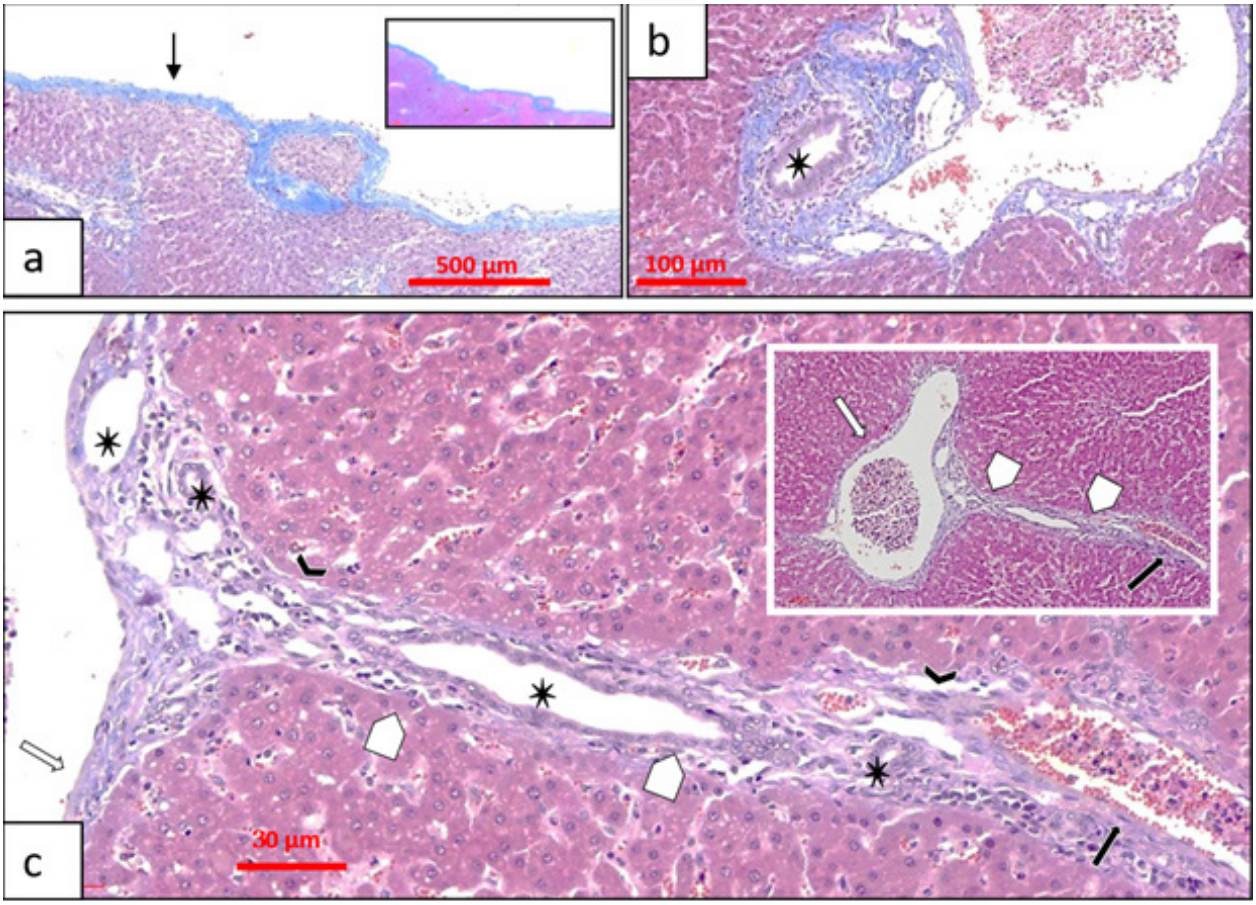

Fig. 4. Sheep Liver. a-c – histotopograms of liver tissue (Masson's trichrome staining); White arrow – hepatic veins; black arrow – portal vein; free arrow – hepatic capsule (Laennec's capsule); pterygoid arrowhead – fissure; black asterisk – bile duct (bile ductule); pentagonal arrow – fibril-like porta-caval fibrous connection

Sheep. In the liver of the sheep, the portal and the caval ports are somewhat separated from each other (the hilum of the portal vein is located more ventrally). In this respect, sheep liver, like cow liver [34], shows more similarity with the human liver. The portal and hepatic veins, respectively, are located at the planes, which are at different angles to each other and intersect spatially.

The structure and location of the connective tissue structures of sheep liver are more similar to the structure and location of connective tissue structures of rat and hen livers than that of porcine liver. Besides, there is a difference: the liver capsule (Laenek's capsule) contains a large amount of type-I collagen and a small amount of type-III collagen and elastic fibers (Fig 4a).

The bile ducts, which have the star-shaped lumens (similarly to hens bile ducts) and are covered with a sheath containing type-III collagen and single elastic fibers, do not directly touch the liver parenchyma but is separated from it with the type-I collagen fibers. The latter borders the liver parenchyma which in turn is covered by the derivate of Laenek's capsule (Fig 4b).

PCFC was detected only in isolated cases. For the most part, these connections are plate-shaped and contain bile ducts, making the sheep liver like the liver of a rat (Fig 4c).

Conclusion. In mammalian and bird livers, the connective tissue sheaths of various thicknesses and compositions around the portal tracts and hepatic veins are interconnected in various ways with each other, the liver capsule and intralobular connective tissue network. This system of connective tissue fibers forms the so-called liver extracellular matrix - the connective tissue skeleton of the liver.

The connective tissue sheaths around the portal tracts and the hepatic veins might be connected to each other in the form of fusion, touching, septum (plate-shaped), or thread-shaped connections when intersecting each other. Such connections form a sturdy extracellular matrix and strengthen the architecture of the liver tissue that helps the organ maintain its integrity under various pathological (including traumas) conditions. Due to the minimal number (virtually absent) of portocaval connective fibrous connections, compared to that of the livers of pigs, sheep, and rats, the hen livers seem to be more vulnerable to mechanical damage.

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SUMMARY

LIVER EXTRACELLULAR MATRIX PECULIARITIES IN MAMMALS AND AVIANS

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Analysis of liver matrix studies makes it obvious that knowledge about the connective tissue skeleton of the liver is not systematized, the terminology is contradictory, and the question of the construction of some components sometimes causes controversy. We set a goal to study the features of the construction of the connective tissue matrix of the liver of various mammals and birds in order to identify and systematize general and specific patterns of this structure.

The liver of mammals with a gallbladder (pigs, sheep) and without a gallbladder (rats) and birds (domestic chickens with a gallbladder) was studied by the methods of anatomical preparation, histology, histochemistry, histotopography, immunohistochemistry, scanning electron microscopy of corrosion replicas and fluorescence microscopy.

In the liver of mammals and birds, connective tissue membranes of various thicknesses and compositions around the portal tracts and hepatic veins are revealed. These membranes are connected in various ways with each other, the liver capsule and the intralobular network of connective tissue and form an extracellular matrix, which strengthens the structure of the liver tissue and helps the organ maintain its integrity in various pathological conditions.

Keywords: portal sheath, hepatic vein sheath, liver matrix, porta-caval fibrous connections, liver capsule.

РЕЗЮМЕ

ОСОБЕННОСТИ ВНЕКЛЕТОЧНОГО МАТРИКСА ПЕЧЕНИ У МЛЕКОПИТАЮЩИХ И ПТИЦ

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Анализ исследований матрикса печени выявил, что знания о соединительнотканном каркасе печени не систематизированы, терминология противоречива, а вопрос о строении того или иного компонента печеночного матрикса вызывает споры.

Целью исследования явилось определение особенностей строения соединительнотканного матрикса печени различных млекопитающих и птиц для выявления и систематизации общих и частных закономерностей этой структуры.

Печень млекопитающих с желчным пузырем (свиньи, овцы) и без желчного пузыря (крысы) и птицы (домашние куры с желчным пузырем) изучали методами анатомического препарирования, гистологии, гистохимии, гистотопографии, иммуногистохимии, сканирующей электронной микроскопии коррозионных реплик и флуоресцентной микроскопии.

В печени млекопитающих и птиц выявляются соединительнотканные оболочки различной толщины и состава вокруг воротных трактов и печеночных вен. Эти оболочки различными способами связаны друг с другом, капсулой печени и внутрилобулярной сетью соединительной ткани, образуя внеклеточный матрикс, который укрепляет структуру ткани печени и помогает органу сохранять целостность при различных патологических состояниях.

რეზიუმე

ღვიძლის ექსტრაცელულური მატრიქსის თავისებურებები ძუძუმწოვრებსა და ფრინველებში

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კვლევების ანალიზი უჩვენებს, რომ მონაცემები ღვიძლის შემაერთებელი ქსოვილის ჩონჩხის შესახებ არ არის სისტემატიზებული, ტერმინოლოგია წინააღმდეგობრივია და ზოგიერთი კომპონენტის სტრუქტურის საკითხი ზოგჯერ ურთიერთსაწინააღმდეგოდ არის წარმოდგენილი.

კვლევის მიზანს წარმოადგენს სხვადასხვა ძუძუმწოვრებისა და ფრინველების ღვიძლის შემაერთებელქსოვილოვანი კარკასის აგებულების თავისებურებების, მისი სტრუქტურის ზოგადი და სპეციფიკური ნიშნების იდენტიფიცირება და სისტემატიზაცია.

ანატომიური პრეპარაციის, ჰისტოლოგიური, ჰისტოქიმიური, ჰისტოტოპოგრაფიული, იმუნოჰისტოქიმიური, კოროზიული ტვიფრების მასკანირებელი ელექტრონული მიკროსკოპიის და ფლუორესცენტული მიკროსკოპიის მეთოდებით შესწავლილია ნაღვლის ბუშტის მქონე (ღორი, ცხვარი) და ნაღვლის ბუშტის არმქონე (ვირთაგვა) ძუძუმწოვრების და ნაღვლის ბუშტის მქონე ფრინველების (მამალი) ღვიძლები.

ძუძუმწოვრებისა და ფრინველების ღვიძლში პორტული ტრაქტებისა და ღვიძლის ვენების გარშემო გამოვლინდა სხვადასხვა სისქის და კომპოზიციის შემაერთებელქსოვილოვანი გარსები, რომლებიც სხვადასხა ფორმით არის დაკავშირებული ერთმანეთთან, ღვიძლის კაფსულასთან და შემაერთებელი ქსოვილის წილაკშიდა ქსელთან და ქმნის ღვიძლის ერთიან მატრიქსს, რომელიც სიმტკიცეს აძლევს ღვიძლის ქსოვილის სტრუქტურას და უზრუნველყოფს ღვიძლის სტრუქტურული მთლიანობის შენარჩუნებას სხვადასხვა პათოლოგიური ზემოქმედების პირობებში.

STRUCTURAL CHANGES IN RATS' LIVER DURING THE FIRST 2 WEEKS FOLLOWING 2/3 PARTIAL HEPATECTOMY

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Liver regeneration followed by liver resection is one of the most frequently studied processes, both in the clinic and in the experiment [1,3,6,7,23,25,26]. Dramatically is increased frequency of liver resection in recent years. This is due, on the one hand, to increased cases of space occupying liver pathologies which were previously considered as inoperative, including through endoscopic intervention [14] and on the other hand, the increase in the frequency of liver resections is associated with widespread transplantations of half liver from a living donor.

According to the data of Eurotransplant International Foundation in 2019 in Europe 116 cases of liver transplantation were performed from living donor. The United Network for Organ Sharing reports 524 liver transplantation from living donor. In total, between 1988 and October 31, 202 7715 liver transplantation were performed in the United States from a living donor.

One of the important characteristics of liver transplantation from a living donor is that, after the operation both, the remnant liver in the donor and half liver transplanted into the recipient are regenerated - by restoring the initial mass and volume of the liver. This characteristic led to the revision of regeneration processes, including given that the regeneration of the transplanted liver takes place under conditions of denervation and delymphatization [2,13,19].

Despite that several hundred papers on liver regeneration are published each year, important questions for regenerative medicine remain unanswered, including such simple question as: Does normal liver differ from regenerated one, and if so, how it differs.

It is known that postanal period of ontogenesis involves the proliferation of both liver lobules and cells, while the liver regeneration happens due to cell proliferation without increasing the number of lobules [18,24].

Our study of both the specimens of the liver and the three-dimensional architecture of its tubular structures and connective tissue spaces has shown that the process of liver regeneration takes place due to/is accompanied by complex morphological changes. The changes concerns both the parenchymal and stromal components of the liver [20,27,28]. In this study comparison of normal and regenerated liver morphologies allowed us to conclude that regeneration of liver mass after resection is due to hepatocyte hypertrophy, changes in their shape and size, sinusoidal dilatation and proliferation, as well as their prolongation, and multiplication of interlobular connective tissue, which causes changing the structure of the lobul - remodeling. Remodeling is also indicated by the difference in the shape and size of hepatocytes from normal in the hepatic acinus zones according to the Rappaport [5,21].

In addition, we have also shown the formation of "megalobules" by the union of adjacent lobules after 2/3 resection in 9-month-regenerated liver, as well as smaller lobules than in normal one. The description of the megalobules is similar with the data of Papp et al., who showed the formation of big surface lobules [4,18]. As for the presence of small lobules in the regenerated liver, similar data could not be found by us. The presence of small lobules requires additional studies to confirm whether, in addition to lobule enlargement and remodeling, they also multiply in the regeneration process.

It has been proven that the increase in rodent liver size and weight after resection ends 7-10 days after surgery, and the recovery of lobules architecture - in 10-14 days [11,17,21]. Although most studies of liver regeneration focus on these dates, even in the acute period after hemihepectomy, the questions we have highlighted above concerning the structural difference between normal and regenerated livers remain unanswered.

Considering the above, we aimed to investigate changes the hepatocyte size and shape and the architecture of the sinusoidal network in the 2-week dynamics after resection 2/3 of the liver.

Material and methods. The experiments were performed on 16 adult male Wistar rats weighing 190-200 grams, who underwent 2/3 hepatectomy. We examined their liver tissue by histological, immunohistochemical, morphometric methods, and the spatial architecture of the sinusoidal capillary network by electron microscopy of the corrosion casts. The study was conducted in 24 hours, 48 hours, 96 hours, 1 week, and 2 weeks after surgery. The resected part of the liver of the same rat was used as a control. Corrosion casts of normal animal liver were taken from the archives left from previous studies [27,28]. The sex and weight of the rats in these studies were similar.

The number and distribution of animals according to the research terms and methods are given in Table 1.

Table 1. Number and distribution of animals in the study group by term and research methods

Groups and terms / Research Methods	Research Group (PH)				
	24 H	**48 H**	**96 H**	**1 week**	**2 weeks**
Histology*	2	2	2	2	2
Immunohistochemistry*	2	2	2	2	2
Morphometry*	2	2	2	2	2
Corrosion Casts	1	1	1	2	1
Sum of animals	**3**	**3**	**3**	**4**	**3**

** - the same animal was used for these methods*

Surgery models. **2/3 Hepatectomy:** The Animal Care Protocol incorporated the recommendations of the National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals to minimize animal pain and / or discomfort, both during and after surgery [9]. Before and after the experiments the animals were kept in comfortable laboratory conditions (22° C, 12 h/12 h, light /dark, 60% humidity, free access to food and water). They had restricted access to the food only on the day before the operation. The operation was performed in fasting state, with general anesthesia with a mask of diethyl ether.

2/3 hepatectomy was performed according to the protocol of Claudia Mitchell & Holger Willenbring, by using double knot method. After opening the abdominal cavity, the liver was mobilized by crossing the liver ligaments. Left lateral lobe (26% of liver) was resected after the first knot which was followed by the second knot and resection of median lobe (38-42% of liver) [16]. Resected parts of liver were examined macromorphological for the exclusion any pathology.

Histology. Liver tissue sections of 3-μm were stained by standard H&E method and studied microscopically with different magnification. (Primo star ZEISS, Jena, Germany) equipped with a digital video camera (ZEN 2.3 SP1).

Immunohistochemistry. For immunohistochemical investigation Keratin-8 antibody (MyBiosourse) was used. Diluted rate 1:200 in 0,01M Phosphate Buffer Saline (PBS) pH7.4 (Sigma Aldrich). The tissue was formaldehyde fixed and a heat mediated antigen retrieval step in citrate buffer was performed. The tissue was then blocked and incubated with the antibody for 2 hours at 22°C. An HRP (расшифровать) conjugated goat anti-rabbit antibody was used as the secondary. Slides were observed and imaged under a light microscope (Primo star ZEISS, Jena, Germany) equipped with a digital video camera (ZEN 2.3 SP1).

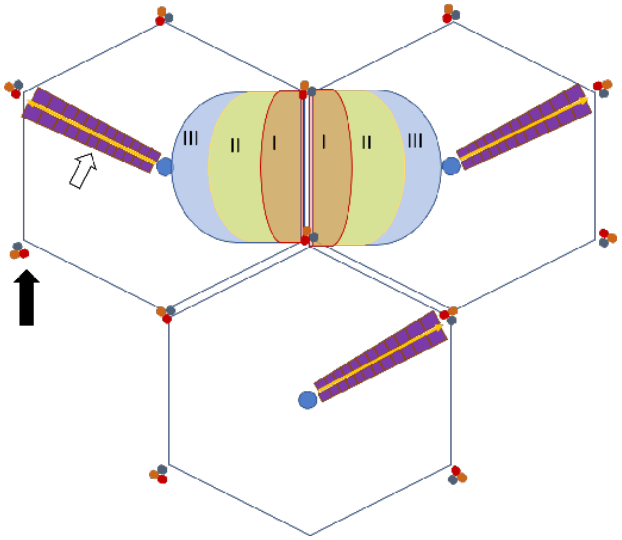

Fig. 1. Liver lobules and acinus. I, II,III – the zones of acinus; White arrow – hepatocytes' plate; Black arrow – portal triad; White arrowhead – central vein

Morphometry. For morphometric analysis, we selected areas similar to those previously described by us (Fig. 1) [27,28], namely:

a) the hepatocytes of the first zone of the hepatic acinus, located near the line connecting the adjacent portal triads (on both sides), corresponding to the perpendicular line from the portal area to the axis connecting the central veins of the adjacent liver lobules. This is the zone that first receives oxygen and nutrients, the dominant process in this area is oxidative metabolism (gluconeogenesis, proteosynthesis) [8,12].

b) the hepatocytes of the third zone of hepatic acinus, located around the central vein, corresponding the top part of portal triad. This is the zone which receive the least amount of oxygen. The reduction processes are predominant in this location – e.g. detoxication [12].

Slides stained with CK8 marker were used for morphometric analysis. CK8 provided good visualization of the hepatocyte membrane and ensured a high accuracy of marking the measurable area.

Hepatocyte area and perimeter measurement were performed on the right lobes of the liver of both the control and study group.

Histological slides were scanned on Motic Digital Slide scanner and morphometry was performed using Motic digital scanner assistant software Motic VM 3.0. The working area was magnified 40 times and the cell membranes were lined up manually because the shape of the hepatocytes did not normally exactly match any of the geometric figures. For morphometric analysis were selected cells with fully visualized membrane and nucleus. 3 samples of I and III zones were selected from each animal. 100 cells in each zone were measured.

Scanning Electron Microscopy (SEM) of corrosion casts

All the conditions and sequences were done as previously described by us [27,28]. To make corrosion casts we used a mixture of benzoyl peroxide, MAYCRYL C.C., powder and Protacryl-M as described in our article. Injectable solidifying mass was injected into rats via portal vein under anesthesia with diethyl ether, which was followed by pre-rinsing of the blood bed with 0.9% saline (rinsing time in 20 ml/min).

We examined corrosion casts with electron microscope JEOL-JSM-6510LV, which allowed the sample to be visualized by analyzing both direct and reflected electron flows in both high and low vacuum conditions. For investigation under high vacuum conditions, corrosion casts were coated with a layer of gold atoms, JEC-3000FC (using Tokyo BOEKI Group, Japan apparatus (vacuum=3.2 Pa, coverage time=180 sec).

Continuous variables are presented in average (min, max, standard deviation). Two-sample t-test was used for comparison of continuous variables. These tests were 2-sided. The P values <0.05 was considered statistically significant. Analysis was performed with SAS version 9.3 software (SAS Institute, Inc., Cary, NC, USA).

Results and discussion. Tables 2 and 3presents perimeter and area of hepatocytes on study and control groups.

24, 48, and 96 hours after liver resection, the area and perimeter of hepatocytes increased in the first and third zones of the acinus compared to normal ($p<0.001$). 1 week after resection, the area and perimeter of hepatocytes in the third zone of the acinus were smaller than normal ($p<0.05$), and the perimeter and area of hepatocytes in the first zone exceeded the normal values ($p=0.05$). In addition, the perimeter and area of hepatocytes in the first and third zones of the acinus were smaller compared to similar data for previous regeneration terms ($p<0.05$). 2 weeks after resection, the area and perimeter of the regenerated liver hepatocytes in the first and third zones of the acinus exceeded the normal values obtained one week after resection ($p<0.001$).

Table 2. Hepatocytes area and perimeter in the I zone of acinus

Terms and zone / Data	Area of I zone (μm²)						Perimeter of I zone (μm)					
	Norma	24 H	48 H	96 H	1 week	2 weeks	Norma	24 H	48 H	96H	1 Week	2 Weeks
Average	255	349	479	315	275	389	62	76	93	74	63	77
Minimum	128	203	228	135	132	219	44	52	65	47	46	53
Maximum	418	780	982	863	734	737	88	106	150	108	92	105
St. Deviation	66	97	160	117	88	120	8	11	16	13	11	12

Table 3. Hepatocytes area and perimeter in the III zone of acinus

Terms and zone / Term data	Area of III zone (μm²)						Perimeter of III zone (μm)					
	Norma	24 H	48 H	96 H	1 week	2 weeks	Norma	24 H	48 H	96H	1 Week	2 Weeks
Average	283	388	400	347	244	379	64	76	77	75	61	74
Minimum	119	224	123	34	34	160	47	54	50	49	42	51
Maximum	523	665	897	654	506	715	90	105	111	103	83	104
St. Deviation	88	95	131	97	76	118	10	11	13	12	9	13

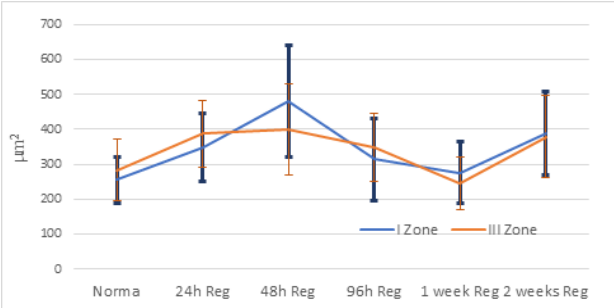

Diagram 1. Area of hepatocytes of I and III zones of acinus

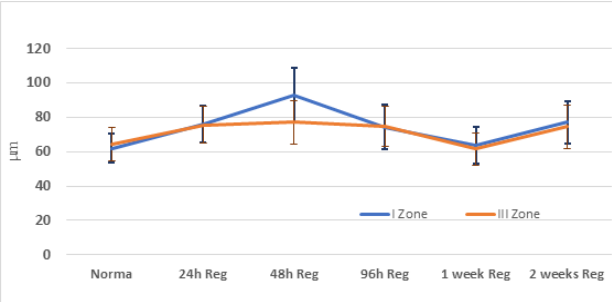

Diagram 2. Perimeter of hepatocytes I and III zones of acinus

Comparison of hepatocyte areas and perimeters by zones at the same terms shows that, at normal and at 24 h of regeneration, the area of hepatocytes in the third zone exceeds the area of hepatocytes in the first zone (p=0.01; p=0.005, respectively), and no significant difference is observed between the perimeters of the hepatocytes (respectively p=0.06; p> 0.9).

The situation is changed at 48 and 96 hours after resection, when the area and perimeter of hepatocytes in the first zone of the acinus exceeded the area and perimeter of hepatocytes in the third zone (p <0.05).

One week after 2/3 liver resection, the area of hepatocytes in the first zone was significant larger than the area of hepatocytes in the third zone (p=0.009), and the difference between perimeters was not significant (p=0.1). However, two weeks later, the area and perimeter of hepatocytes in the first zone of acinus do not differ from the area and perimeter of hepatocytes in the third zone (p=0.5; p=0.2). All data are presented graphically in Diagrams 1 and 2.

By the histological examination of the normal rat liver can often identify lobules where the hepatocytes are arranged radially, in the form of plates of one or two hepatocytes, between which the sinusoids are more or less equal in size (Fig. 2A, 3A).

On the 24th and 48th hours after 2/3 hepatectomy, it is difficult to identify the lobules, and the lobules whose outline can be identified are increased. The radial arrangement and architecture of the hepatocyte plates are disordered. They are replaced by conglomerates of liver cells and sinusoidal capillaries. In some areas of these conglomerates, zones without sinusoids, as well as with sinusoids of different sizes and shapes, are identified. In addition, some sinusoids are sharply widened. The typical configuration of the cytoplasmic membrane of hepatocytes are changed, cytoplasmic protrusions (procesus) appear on some hepatocytes. Multiple mitotic figures (Fig. 2B, C, 3B, C) are observed On the 48th h of regeneration. On the 96th h of regeneration mitotic figures are found in unit quantities. Part of hepatocytes undergo destructive-necrotic changes. Such necrotic hepatocytes are often bordered by binuclated, large, or mitotic hepatocytes. Necrotic hepatocytes are also often found in so-called, bloodless areas (Fig.s 2D; 3D). 1 week after regeneration, the liver tissue returns to a more or less typical architecture, and the size of the lobules that can be identified on histological preparations is larger comparing to normal. Part of the sinusoids is dilated, and in some areas there are markedly irregular sinusoids with branching. The plasma membrane of hepatocytes surrounding such sinusoids is also abruptly irregular, sometimes so much that the liver cells have a star-like shape (Fig. 2E; 3E; Fig. 5). On the 24th , 48th , and 96th hours of regeneration, hepatocytes differ markedly in size from normal hepatocytes. Typical form of the normal hepatocyte (Fig. 2A, 3A) are replaced by hepatocytes with dramatically different shapes and sizes, which are connected to each other by an unusually shaped

plasma membrane protrusion so that the histological picture of whole section resembles the puzzle construction (Fig. 2B, C, D, E, F; 3B, C, D, E, F). Connection of hepatocytes through these new "growths" (protrusions) indicates formations of new connections, and remodeling of hepatocyte cords, as it is shown in the case of changes in aortic endothelial cell under pressure [10].

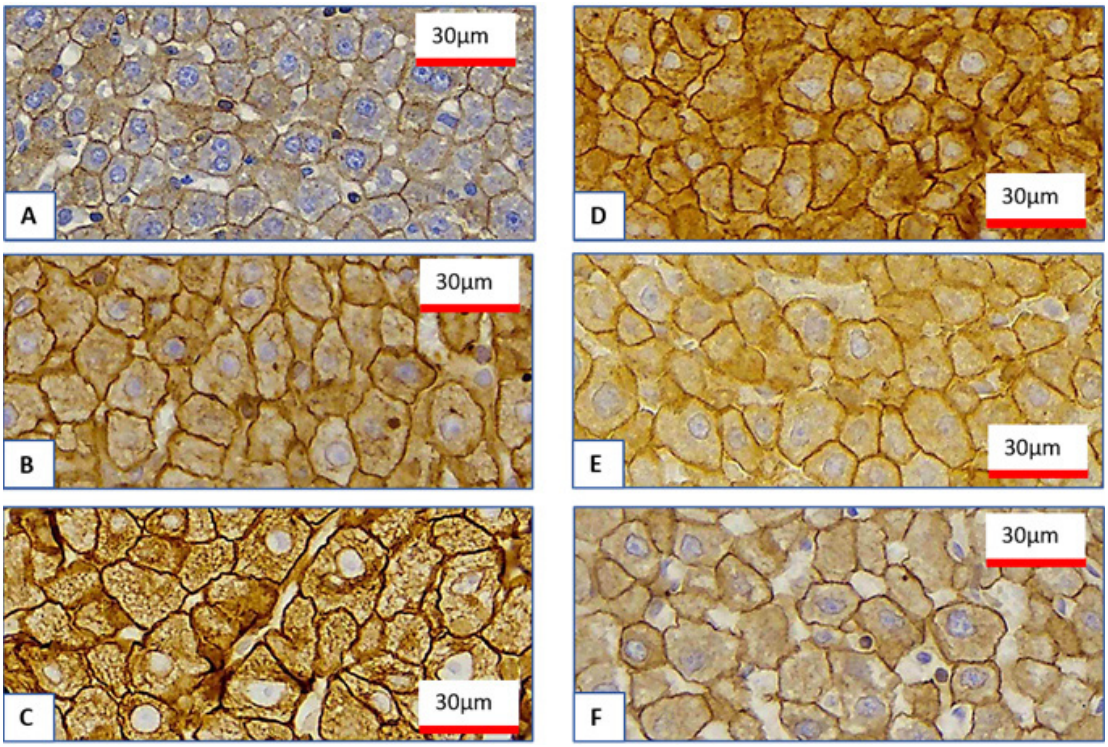

Fig. 2. Hepatocytes in the I zone of acinus; A - Control; B – 24h after PH; C – 48h after PH; D – 96h after PH; E – 1week after PH; F – 2 weeks after PH; Marked with CK8; X1000

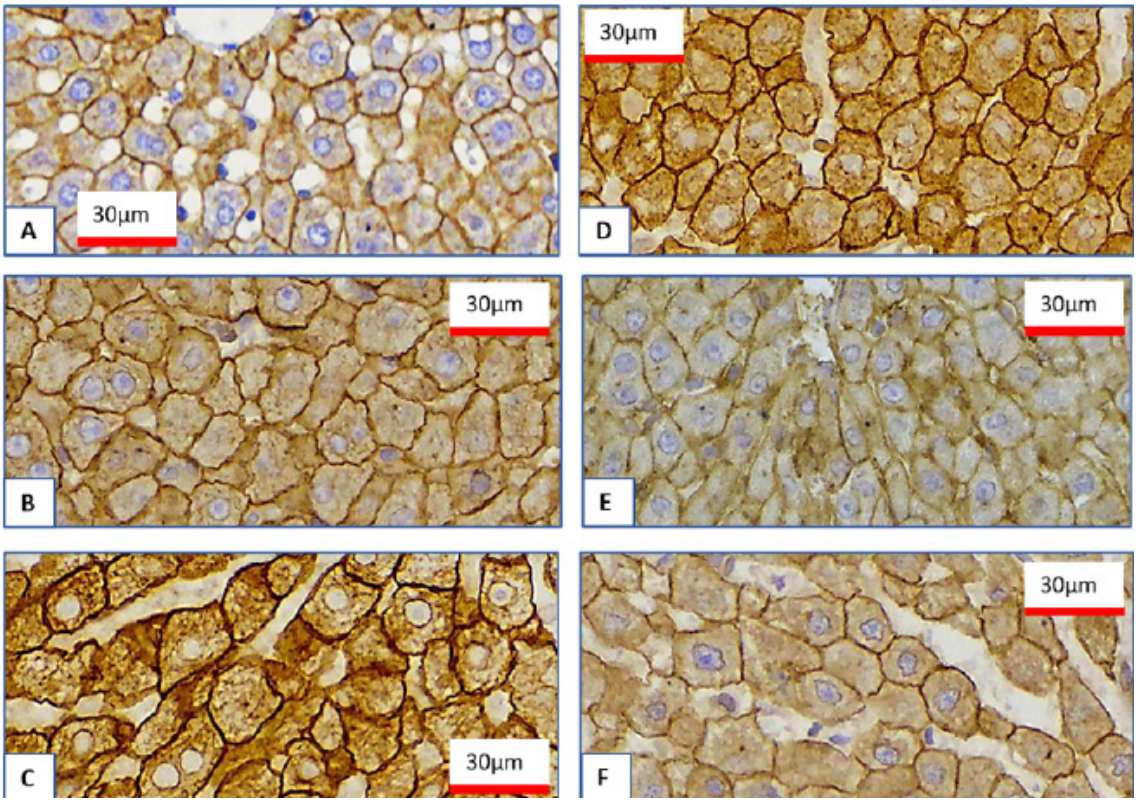

Fig. 3. Hepatocytes in the III zone of acinus; A - Normal Liver; B – 24h after PH; C – 48h after PH; D – 96h after PH; E – 1week after PH; F – 2 weeks after PH; Marked with CK8; X1000

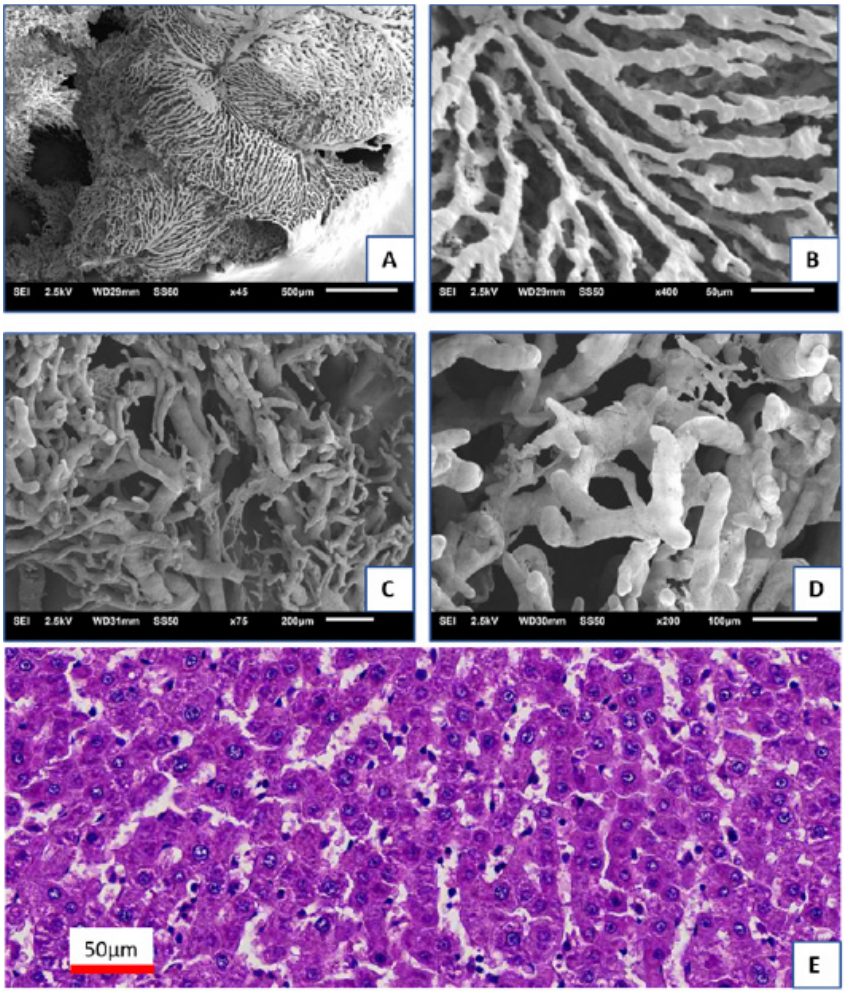

Fig. 4. SEM of Corrosion cast of liver regeneration after 1 week of PH;
A – sinusoidal network of adjusted lobules. Injection replicas of the sinusoids with different forms and diameters.
B- Rough surface injection replicas of thin, zigzag-like form sinusoids.
C,D – sprouting of hepatic vein tributaries and large sinusoids. E – H&E stain; Zigzag-like form sinusoids (corresponds with B)

It is noteworthy that within a week after regeneration in some areas there is intense formation of sinusoid capillaries and an abundance of small tributaries of the hepatic veins (central and sublobular veins) against the background of the small amount of portal triads.

SEM examination of corrosion cats of the same term reveals a network of sinusoids that spatially lines lobules of different shapes and sizes, including those that appear to be a combination of two "normal" lobules (megalobules) (Fig. 4A). Sometimes the diameter of sinusoids is markedly different. Particularly the superficial sinusoids. In some areas, small (narrow) diameter casts of sinusoids are observed, which have an irregular rough surface with small bud protrusions, which gives the contour of these casts a zigzag shape (Fig. 4B). These casts correspond to the sinusoids observed in some slides prepared on the same term and stained by H&E. SEM of corrosion casts reveals the replicas of the hepatic vein tributaries and associated with them large sinusoids which with the typical features of vascular sprouting. Such sprouting casts sometimes anastomose to each other (Fig. 4 C, D).

After 2 weeks of liver resection, the number of areas whose construction looks like normal increases. In addition, areas with hepatocytes with cytoplasmic growths (protrusion) and sinusoids of different diameters are still detected. Regenerative nodules without sinusoids indicating that the sinusoidalization process is not complete.

Changes the data of hepatocyte area and perimeter obtained by us are not characterized by a single common tendency. Taking into account, that hepatocytes of a strange (non-standard) shape appear with a kind of cytoplasmic protrusions (Fig. 5), we must assume that not only the vascular network and, consequently, the shapes and sizes of the lobules, but also the population of hepatocytes are subject to transformation. Comparing the current data obtained by morphometry of hepatocytes after 2/3 hepatectomy, with the similar data obtained 9 months after 2/3 hepatectomy, previously published by us, it turns out that they are also different from each other [28]. This gives us reason to assume that structural transformation of the liver is a prolonged process after 2/3 resection. These data corrects the statement that liver regeneration processes in rodents after resection of 2/3 ends in 7 – 10 [11,17,22].

Based on the results of our research, we consider that such formulation is more correct: after 2/3 resection, the liver regenerates and regains its mass and volume in 7-10 days, although the transformation of both, its cellular and vascular structures, which in turn leads to spatial transformation of liver cells, continues for long periods from resection.

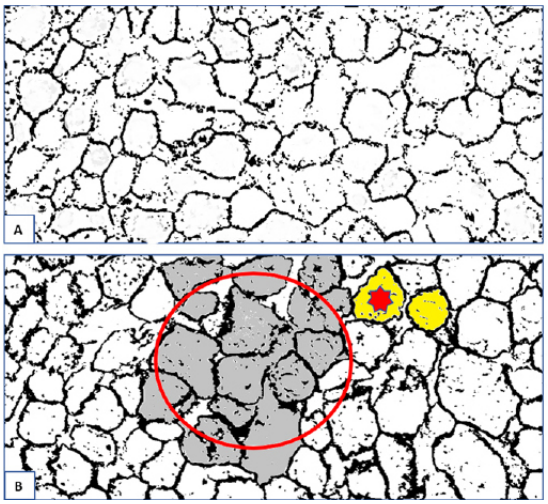

Fig. 5. Hepatocyte forms changing.

A) Normal Liver. Scanned image. Marked with CK8; edited by ImageJ software X1000

B) Liver after 1 week Regeneration. Marked with CK8; edited by ImageJ software X1000. Hepatocytes with dramatically different shapes and sizes, which are connected to each other by an unusually shaped plasma membrane protrusion so that the histological picture of whole section resembles the puzzle construction (bordered by red circle); Hepatocytes with zigzag-like membrane (asterisk)

It is under the question, whether the permanent transformation of liver architecture is caused only by 2/3 hepatectomy or it is a typical phenomenon for the liver that occurs throughout ontogenesis. During the period of ontogenesis, the liver (as well as the whole organism) increases in volume and weight, and this increase is associated with the proliferation of liver lobules [18]. After completion of postnatal growth, in the dynamics of ontogenesis, confirmation or rejection of possible changes in liver architecture would be important for the study of liver structure, as a whole, and as an individual component of the organ.

Sprouting of the hepatic vein tributaries (Fig. 4C, D) detected on corrosion casts confirms that the proliferative processes are not complete and therefore the liver/lobules remodeling process continues. The sprouting of the above-mentioned venules corresponds to areas quite commonly found on histological slides, where exists a lot of central veins and sublobular veins without a corresponding number of triads, this does not fit with the classical description of rat liver. In addition, a similar proliferation of veins may be some indication of the formation of new lobules.

Conclusion. The process of regeneration of rat liver does not end in one or two weeks. Despite the recovery of liver volume and mass, which is mainly based on hepatocyte mitoses, the regenerated liver undergoes a permanent process of transformation of hepatocyte shape and size, as well as the transformation of the vascular network. New intercellular connections are formed, including with the involvement of atypical membrane protrusions of deformed neighboring hepatocytes. The vascular network also undergoes transformation - by changing the shape and size of existing structures and by forming new sinusoidal capillaries and venules.

These transformations underlie changes in the spatial architecture of the liver lobules.

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SUMMARY

STRUCTURAL CHANGES IN RATS' LIVER DURING THE FIRST 2 WEEKS FOLLOWING 2/3 PARTIAL HEPATECTOMY

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Aim of study - Investigation of changes in hepatocyte size and shape and architecture of the sinusoidal network in 2-week dynamics after resection 2/3 of the liver.

The experiments were performed on 16 adult male Wistar rats weighing 190-200 grams who underwent 2/3 resection of liver, while a resected portion of the liver of the same rat was considered as a control. We examined liver tissue by histological, immunohistochemical, morphometrical methods, and the architecture of the sinusoidal capillary network by electron microscopy of corrosion casts. The study was conducted in 24 hours, 48 hours, 96 hours, 1 week, and 2 weeks after surgery.

The shape and size of the hepatocytes in the first and third zones of the liver acinus change with the term of the experiment. With changes in the shape and size of hepatocytes, new intercellular connections are formed, including with the involvement of atypical membrane protrusions of deformed neighboring hepatocytes.

One week after regeneration, electron microscopic examination of corrosion casts reveals a network of sinusoids that spatially define lobules of different shapes and sizes, including those that appear to be a combination of two "normal" lobules. Superficial sinusoids are often markedly dilated (up to 25 μm). In addition, small-diameter (6-7μm) sinusoidal casts with a rough surface and small bud-shaped protrusions are observed in some areas, giving the line of this a zigzag shape. The existence of hepatic vein tributaries and associated with them large sinusoids, found In single areas, reveals the characteristic feature of vascular sprouting.

Based on the data obtained, it can be assumed that despite the recovery of liver mass, the regeneration process is not complete. Regenerated liver undergoes a permanent process of transformation of hepatocytes' shape and size, as well as the transformation of the vascular network, which is the basis for changes in the spatial architecture of the liver lobules.

Keywords: 2/3 liver resection; Liver regeneration; Corrosion casts; Sinusoidal network transformation; Hepatocytes morphometry.

РЕЗЮМЕ

СТРУКТУРНЫЕ ИЗМЕНЕНИЯ ПЕЧЕНИ КРЫС В ТЕЧЕНИЕ ПЕРВЫХ 2-НЕДЕЛЬ ПОСЛЕ 2/3 ГЕПАТЭКТОМИИ

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Цель исследования - изучение изменений размеров и формы гепатоцитов и архитектоники сети синусоидов в течение 2-недель после резекции 2/3 печени.

Эксперименты выполнены на 16 крысах самцах линии Wistar весом 190-200 грамм, у которых выполнена частичная гепатэктомия. Удаленная часть печени проанализирована в качестве контроля для каждого животного. Ткань печени спустя 24, 48, 96 часов и через 1 и 2 недели изучена с помощью гистологических, иммуногистологических и морфометрических методов; архитектоника синусоидных капилляров исследована с помощью сканирующей электронной микроскопии коррозионных препаратов.

Форма и размеры гепатоцитов первой и третьей зоны печеночных ацинусов изменяются в течение всего срока наблюдений. Изменение формы и размеров гепатоцитов приводит к формированию новых межклеточных контактов, которые в ряде случаев образуются благодаря атипичным отросткам деформированных соседних клеток. Спустя неделю после гепатэктомии электронно-микроскопическое исследование коррозионных препаратов выявило сеть синусоидов, которые располагаются внутри долек различной формы и размеров. В некоторых случаях создается впечатление, что дольки аномальной формы и размеров образованы комбинацией двух «нормальных» долек. Поверхностные синусоиды часто заметно расширены (до 25 мкм). На некоторых участках наблюдаются слепки синусоидов малого диаметра (6-7 мкм) с шероховатой поверхностью и небольшими выступами в форме бутона, что придает им зигзагообразную форму. В ряде зон обнаруживается наличие притоков печеночных вен и связанных с ними синусоидов большого диаметра, что является признаком сосудистого разрастания.

Полученные данные позволяют предположить, что, несмотря на восстановление массы печени, процессы регенерации не завершаются. В регенерирующей печени продолжаются перманентные процессы трансформации формы и размеров гепатоцитов, а также перестройки сети сосудов, которые лежат в основе изменений пространственной архитектуры долек печени.

რეზიუმე

ვირთაგვის ღვიძლის სტრუქტურული ცვლილებები 2/3 ჰეპატექტომიიდან პირველი 2 კვირის განმავლობაში

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[2] თსუ, ალექსანდრე ნათიშვილის სახ. მორფოლოგიის ინსტიტუტი; [3]ფედერალური სამედიცინო ბიოლოგიური სააგენტო, ფიზიკურ-ქიმიური მედიცინის ფედერალური კვლევითი და კლინიკური ცენტრი, მოსკოვი, რუსეთი

კვლევის მიზანს წარმოადგენდა ღვიძლის 2/3-ის რეზექციის შემდეგ ღვიძლის რეგენერაციის პროცესში ჰეპატოციტთა ზომის, ფორმის და სინუსოიდთა ქსელის სივრცული არქიტექტონიკის ცვლილებების გამოკვლევა 2-კვირიან დინამიკაში.

ექსპერიმენტები ჩატარდა Wistar-ის ჯიშის 16 ზრდასრულ მამრ ვირთაგვაზე, წონით 190-200 გრამი, რომელთაც ჩაუტარდა პარციალური ჰეპატექტომია; მათ კონტროლად აღებული იყო იმავე ვირთაგვას ღვიძლის რეზეცირებული ნაწილი. ღვიძლის ქსოვილის გამოკვლევა ჩატარდა ჰისტოლოგიური, იმუნოჰისტოქიმიური, მორფომეტრიული მეთოდებით; სინუსოიდური კაპილარების ქსელის სივრცული არქიტექტონიკა - კოროზიული ტვიფრების მასკანირებელი ელექტრონული მიკროსკოპიით. კვლევა ჩატარდა ოპერაციიდან 24, 48 და 96 საათის, 1 და 2 კვირის შემდეგ.

ექსპერიმენტის ვადებთან ერთად ცვალებადობა განიცადა ღვიძლის აცინუსის პირველი და მესამე ზონების ჰეპატოციტების ფორმისა და ზომის ურთიერთშეფარდებამ. ჰეპატოციტების ფორმისა და ზომის ცვლილებებთან ერთად ჩამოყალიბდა ახალი უჯრედშორისი კავშირები, მათ შორის ფორმაშეცვლილი მეზობელი ჰეპატოციტების ატიპიური მემბრანული მორჩების ჩართულობით.

რეგენერაციის 1 კვირის შემდეგ კოროზიული პრეპარატების მასკანირებელი ელექტრონული მიკროსკოპით გამოკვლევისას გამოვლინდა სინუსოიდთა ქსელი, რომელიც სივრცულად საზღვრავდა სხვადასხვა ფორმისა და ზომის წილაკებს, მათ შორის ისეთებსაც, რომლებიც, თითქოს შექმნილია ორი „ნორმული“ წილაკის გაერთიანებით. ზედაპირულად მდებარე სინოსოიდები ხშირად იყო მკვეთრად გაგანიერებული (25 მკმ-მდე). ამასთანავე, ცალკეულ უბნებში აღინიშნებოდა მცირე დიამეტრის (6-7 მკმ) სინუსოიდთა ტვიფრები ხორკლიანი ზედაპირით და მცირე ზომის კვირტისებური წანაზარდებით, რაც ამ ტვიფრების კონტურს ზიგზაგისებურ ფორმას აძლევს. ცალკეულ უბნებში აღინიშნა ღვიძლის ვენების შენაკადებისა და მათთან დაკავშირებული მსხვილი სინუსოიდების ისეთი ტვიფრები, როგორებიც დამახასიათებელია სისხლძარღვთა სპრუტინგისთვის. მიღებული მონაცემების საფუძველზე ავტორები გამოთქვამენ ვარაუდს, რომ მიუხედავად ღვიძლის მასის აღდგენისა, რეგენერაციის პროცესი არ სრულდება. რეგენერირებულ ღვიძლში მიმდინარეობს ჰეპატოციტთა ფორმისა და ზომის, ასევე სისხლძარღვოვანი ქსელის ტრანსფორმაციის პერმანენტული პროცესი, რაც საფუძვლად უდევს ღვიძლის წილაკების სივრცული არქიტექტონიკის ცვლილებებს.

CORRELATION OF THYROID AUTOIMMUNITY WITH ATHEROSCLEROSIS EVALUATION IN HASHIMOTO'S THYROIDITIS

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According of clinical and scientific research result, the thyroid gland dysfunction (hypothyroidism) play significant role in development of dyslipidemia, atherosclerosis (At) and hence the coronary heart disease (CHD) pathogenesis.

Accumulating results of numerous cross-sectional epidemiological investigations indicate that among other important risk factors, the hypothyroidism and high serum TSH levels are more pronounced cause of endothelial dysfunction, histomorphological changes of large vessels' wall and impact on the mechanisms of cholesterol metabolism. McLeod 2013 [1], based on the large meta-analysis study [2], suggested a causal relationship between autoimmune thyroid disease and atherosclerosis [3-5].

By 2019 ESC/EAS Guideline [6], except of the traditional risk factors, such as the dyslipidemia - high level of Total cholesterol (TC), low density lipoprotein cholesterol (LDLC), triglycerides and low level of high density lipoprotein cholesterol (HDLC) - noted the high importance of carotid and femoral intima- media thickness (IMT) for presence of atherosclerosis in patients with CHD and subclinical hypothyroidism (SH) [7-12].

The study region – Georgia (South Caucasus) is iodine-deficient area with a high prevalence of iodine-deficiency-related disease, such as endemic goiter, thyroid nodules, Hashimoto thyroiditis (HT) - most common cause of primary hypothyroidism [1, 13-15]. Due to the substantial changes in lipid metabolism, these conditions increase high-risk morphological features of atherosclerosis [16, 17]. Against this background, hypercholesterolemia has a direct relationship and the impacts on the dynamics of histomorphological changes in the Hashimoto's thyroid parenchyma; there are significant debates regarding the aim on which the present study is mainly concentrated.

Recently, the relationship between subclinical hypothyroidism (SH) and cardiovascular diseases has been one of the most popular topics. There is still some controversy concerning the cardiovascular impact of SH and management protocols.

The aim of the present study is to investigate the putative association between Hashimoto thyroiditis parenchyma changes and At cardiovascular disease (CHD) clinical characteristics focusing on the causal connection between thyroid function indexes, the lipid profile with follicular epithelia's molecular biology details.

Material and methods. We investigated the patients in Georgian National Center of Internal Medicine and Tbilisi State University affiliated Hospitals (Departments of cardiology, surgery and pathology). Present study was reviewed and deemed exempt from written informed consent by the Ethics committee and Board of medical sciences at Tbilisi State University based on Helsinki-ethical principles declaration for medical research [18].

To reach the planed goal we investigated 52 patients (female), which had undergone total thyroidectomy, lobectomy. In the research basic groups (I and II) were included the patients (pts) with Hashimoto thyroiditis (HT) - 28 pts, and HT with atherosclerosis - 24 pts. For underling the significance of HT in atherosclerosis patients with atherosclerosis (without HT) - 27 pts were included in control (group III).

The diagnosis of atherosclerosis were established by 2019 ESC/EAS criteria - confirmed ACVD (CHD, carotid and femoral arteries atherosclerosis) by using ECG, echocardiography, stress testes, carotid and femoral arteries ultrasonography and in some cases coronarography. The diagnosis of HT were established by TSH, FT4, FT3, anti-TPO tests and confirmed in postoperative specimens histology.

The exclusion criteria were: the patients having III-IV functional class (by Canadian Cardiovascular Society grading of angina pectoris) and unstable angina pectoris, heart failure III-IV (by NYHA classification), arterial hypertension grade 1, 2, 3 (by ESC/ESH guideline, 2018) [19], diabetes mellitus, hepatic and renal failure.

For all studying patients the following analysis was provided: lipid profile, TSH, FT4, Anti TPO; carotid, femoral, thyroid gland ultrasonography.

Laboratory tests

Thyroid hormones and anti TPO

Subclinical hypothyroidism (SH) is characterized by normal serum free T_4 and free T_3 levels and increased serum TSH levels.

Patients involved in the study underwent TSH by the enzyme-linked immunosorbent assay (ELISA) methods name "SANDWICH"-96, well plate, source-serum, venous blood, plasma, IU/ml 0-35 IU/ml, free thyroxine testing, and antibody titer to thyroid peroxidase [20].

Thyroid markers reference range: TSH 0.3-4.2 mIU/L, FT4 0.9-1.7 ng/dL, Anti-TPO <9.0 IU/mL, Anti-Tg<4.0 IU/mL [21, 22].

Thyroid disease categorization and thyroid function index - TSH and FT4 respectively:

Subclinical hypothyroidism > 4.2 mIU/L and0.9-1.7 ng/dL
Subclinical hyperthyroidism < 0.3 mIU/Land0.9-1.7 ng/dL
Overt hypothyroidism > 4.2 mIU/Land< 0.9 ng/dL
Overt hyperthyroidism < 0.3 mIU/L and> 1.7 ng/dL

Lipids profile:

Blood samples were taken after 13 hour fasting. Lipid spectrum was studied in blood serum using "Janway" spectrometry. The quantitative determination of total cholesterol (TC) was performed triglycerides (TG) were determined by the enzyme method, while the content of high density lipoprotein-cholesterol (HDLC): low density lipoprotein - cholesterol (LDLC) and very low density lipoprotein - cholesterol (VLDLC) were determined after the precipitation of low density lipoprotein -cholesterol using BIOLABO, France reactive. LDLC were calculated by Friedwald. The main criteria were: total cholesterol (TC)>160mg/dl, low density lipoprotein - cholesterol (LDLC)>100mg/dl, high density lipoprotein - cholesterol (HDLC) 150mg/dl.

Ultrasound diagnostically methods

Echocardiography

LV mass (LVM) calculations have been made using linear measurements derived from 2D targeted M-mode. LVM estimated by the ASE-recommended formula (from LV linear dimensions): LVM= 0.8 {1.04[(LVIDd + PWTd + SWTd) 3 - (LVIDd)3]}+ 0.6 gwhere PWTd and SWTd are posterior wall thickness at end diastole and septal wall thickness at end diasto-

le, respectively. LVIDd (LV internal diastolic dimension. The indexation of LVM (g/m2) determined in accordance with Height (m) and Body surface area (BSA) m2.

Carotid and femoral arteries ultrasonography

Carotid and femoral arteries intima-media thicknesses were investigated by high-resolution ultrasonography on sonoscope TOSHIBA-SSH 140-A by 5 MHz and 7, 5 MHz linear transducers. The degree of carotid stenosis was determined in transversal and longitudinal sections. The intima-media complexes and atherosclerotic plaques height was measured by the triplex scanning method**.** Carotid arteries intima-media thickness (IMT) was defined from bifurcation 20 mm proximally and 30 mm distally [23]. IMT normal value is <1 mm.

Ultrasonography of the thyroid gland

Ultrasonographic examinations were performed on a TOSHIBA SSH-140-A scanner with 5, 7, and 3.5 MHz transmissions. Examination of the thyroid gland used B to assess the thickness, width, length, and size of the thyroid gland using the appropriate formula (thickness × width × length × 0.479) to assess the structure, surface condition, diffuse and focal changes.

Histological examination

All patients provided written informed consents. This study protocol was approved by the ethics committee of medical sciences at Tbilisi State University based on Helsinki-ethical principles declaration for medical research [18]. The research database included postoperative surgical pathology material obtained from patients with thyroiditis who had undergone total thyroidectomy, lobectomy, and partial resection of the thyroid gland. The pathology material was received from Surgical Units of Tbilisi and West Georgia National Center of Interventional Medicine. Both retrospective data (for the years 2014) as well as prospective material (for the years 2918-2019) were analyzed. Basically, thyroidectomies in the I and II groups of patients were performed for the following reasons: a. patients with bilateral or multiple nodules or symptoms of neck or throat compression, or enlargement during follow-up and b. clinical and physical data indicated for removal.

The diagnosis of HT was based on the level in serum anti peroxidase level - 186 (63-438), TSH, FT4 range and histological findings.

For histological examination of thyroid operative materials, the sliced sections were stained with routine Hematoxylin and Eosin (H&E). Formalin-fixed paraffin embedded (FFPE) tissue sections were routinely processed and stained with hematoxylin and eosin. Immunohistochemical (IHC) staining was performed on FFPE tissue sections with antibodies against the following markers: 1. S100 Protein (clone RTU-S100p Polyclone Antibodies, Biogenex, USA), because Hürthle cells reaction is most remarkable in HT disorders; and 2. p63 (clone 7JUL, Leica, UK), which is a p53 gene family at 3q27-29 homologue nuclear transcription factor. Three of p63 isoforms encode proteins that transactivate on p53 activity and induct cell into apoptosis. The other three isoforms encode proteins, which have inhibitory effect on p53 activity [24]; in our cases, p63 is important to detect oxyphilic metaplasia of thyroid follicular epithelium. As positive control Palatine Tonsils lymphoid tissue specimens were used.

FFPE sections were fixed on poly-L-lysine-coated glass slides and prepared as follows: 1) deparaffinization, rehydration and incubation for 20 minutes in 3% H_2O_2; 2) Immersion in phosphate-buffered saline (PBS) for 20 min; 3) Antigen retrieval in the microwave (600 W) for 20 min, followed by cooling in citrate buffer (0.01 m, pH 6.0). Specimens were incubated with the primary antibodies for 1 hour at room temperature. After that was washed three times with PBS at room temperature. Hematoxylin is used for nuclei counterstaining. All procedures were carried out in compliance with antibodies manufacturers' protocols (Bio Genex, USA; Leica, UK).

Histology slides were reviewed by two pathologists (L. G., T. G.). We used the 2015 American Thyroid Association management guidelines [21, 22].

The statistical analysis was performed using Microsoft Excel 7.0, SPSS-20 version and Mann–Whitney U–test. M±SD (M-mean SD-standard deviation) was calculated. Student-t test was used for the analysis of the data obtained for the groups, Fisher's F criterion for comparing dispersions Differences were considered statistically significant when "p" value was less than 5% ($p < 0.05$). Correlation was tested according to the Pearson's correlation. Comparisons between groups and factors were made using Multivariable linear regression and analysis to evaluate independent risk factors (TSH, IMT, demographic variables (age and gender)) [3, 5, 7]. The coefficient of reliability was calculated by t-s statistics for two different averages and F-statistics. The results of the study were recorded in tables and diagrams.

Results and discussion. The confidence of our results is based on the following points:

1. The study groups do not differ by age, BMI or numbers of patients, which excludes influences in the comparison of Lipid spectrum, TSH and FT4 levels.

2. The clinical characters similarity of the study groups of HT+At (group II) and At (group III) permits to underline the TSH responsibility on the development of dyslipidemia (Fig. 1).

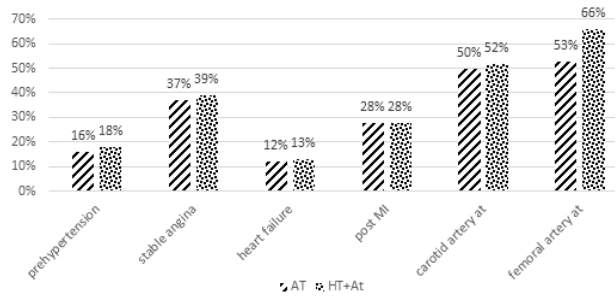

Fig. 1. Clinical characteristic of patients in group II (At+HT) and group III (At)

The clinical characters of the group III (AT) vs group II (AT+HT) are presented in Figure 1. By the analyses of the diagrams 1 and 2 there is no significant differences of group II vs group III patients clinical characteristics that describe the severity of At (Hypertension -16 % vs 18%; stable angina 37% vs 39%; HF 12% vs 13%; post- Myocardial Infarction (MI) 28% vs 28%; carotid artery At), except of femoral artery At - 53% vs 66%.

Analysis of the thyroid gland's functional tests - TSH and FT4, revealed statistically significant differences ($P<0.001$) between Anti-TPO negative group III (patients with At) (TSH:1.2±0.3mIU/L; FT4: 1.2±0.3 ng/dl) and Anti-TPO positive two groups: group I (patients with HT) (TSH:6.0±1.6mIU/L; FT4: 0.98±0.15 ng/dl) and group II (patients with HT+At) (TSH:5.80±1.7mIU/L). There was not thyroid gland's functional tests any differences between group I and II patients as we don't reveal statistical reliable differences of FT4 (P2-3 >0.2) level between group II patients (FT4: 1.1±0.2 ng/dl) and group III patients (FT4: 1.2±0.3 ng/dl) (Table 1).

Table 1. Summary of Baseline Characteristics for Patients With HT, HT+At and At

Groups		Age	BMI	TSH	FT4	TC	LDLC	HDLC	TG
HT (I)	M	62.8	27.4	6	0.98	246.2	172.1	49.3	123.9
	StD	1.2	1.2	1.6	0.15	25.5	23.3	6.1	26.2
HT+At(II)	M	64.6	27.6	5.8	1.1	276.6	204.1	41.3	155.8
	StD	3.8	1	1.7	0.2	11.7	14	6.5	23.6
AT (III)	M	*63.0*	*27.1*	*1.2*	*1.2*	*211.1*	*139.4*	*42.9*	*150.0*
	StD	*3.0*	*1.0*	*0.3*	*0.3*	*29.6*	*29.7*	*5.0*	*22.9*
				TSH	FT4	TC	LDLC	HDLC	TG
			p1-2	0.3	0.2	0.001	0.001	0.001	0.001
			p1-3	0.001	0.001	0.001	0.001	0.001	0.0002
			p2-3	0.001	0.2	0.001	0.001	0.001	0.001

HT- Hashimoto Thyroiditis; At-atherosclerosis; BMI (kg/m2)- Body mass index, TC (mg/dl) –Total Cholesterol; LDLC (mg/dl) –Low density Lipoprotein Cholesterol; HDLC (mg/dl) – High density Lipoprotein Cholesterol; Triglycerides (mg/dl); TSH – (mIU/L; FT4 (ng/dL);; Anti-TPO (IU/mL)

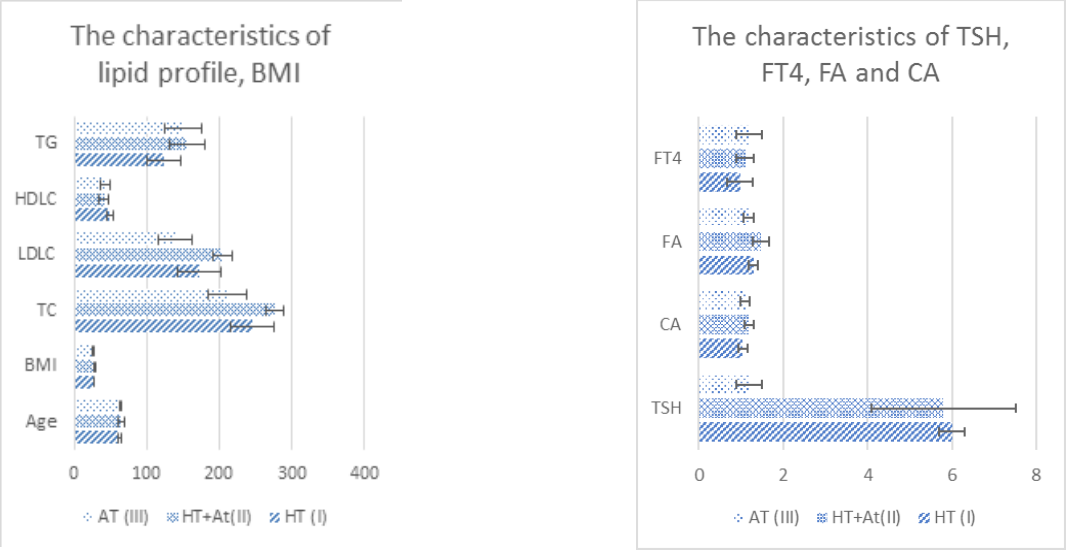

Fig. 2. The characteristics of lipid profile, BMI, TSH, FT4, FA and CA in the study groups

Table 2. CA and FA IMT in study groups

Atherosclerosis		HT(I)	HT+AT(II	At (III)		
CA (mm)	M	1.04	1.19	1.1	p1-2	0.001
	SD	0.09	0.1	0.11	p1-3	0.03
					p2-3	0.0004
FA (mm)	M	1.29	1.47	1.18	p1-2	0.001
	SD	0.13	0.2	0.12	p1-3	0.002
					p2-3	0.0001

Lipid spectre demonstrates more atherogenic changes in II group (HT+At) patients (TC 276,6±11.7.5 mg/dl; LDLC 204,1± 14.0mg/dl; TG 155.8±23.6mg/dl; HDL 41.3±6.5 mg/dl), than in patients with At, but without HT (group III) (TC 211.1±29.6 mg/dl; LDLC 139.4±29.7mg/dl; TG 123.9± 22.9 mg/dl; HDL 49.3± 6.1 mg/dl).

TSH and anti-TPO are important in the development of atherosclerosis as indicated by correlation with atherogenic lipid levels (TC 246, 2±25.5 mg/dl; LDLC 211.1± 29.7mg/dl; TG 150.0 ± 22.9 mg/dl; HDL 42.9 ±5.0 mg/dl), increasing in patients with HT (group I). However, in II group (HT+At) LDLC 276.6± 11.7 mg/dl; TG 155.8 ± 23.6mg/dl; HDL 41.3 ±6.5 mg/dl demonstrate elevation of the same data according to a linear relationship between thyroid function index and lipid profile (Tab.1), respecting BMI and age factors.

The CA (carotid artery) intima-media complex thickening is more expressed in group II patients (1,19±0,1 mm) and statistically significantly differs (p2-3<0.0004) as from group II patients indices (1,1±0,11 mm) as from group I patients indices (1,04±0,09 mm). Also, the statistical reliable between group I and group III indices (p1-3<0.03) were observed (Fig. 2).

There were statistically reliable differences between group II patients FA (femoral artery) intima-media complex indicator (1,47±0,2 mm) with group III atherosclerotic patients without HT (1,18±0,12 mm) p2-3<0.0001 and group I patients with HT (1.29 ±0,13 mm) p1-3 <0.0001 as well between group I patients with group III patients (p<0,001) (Fig.2, Table 2).

These results are derived from linear regression data between serum TSH levels and key diagnostic parameters confirming atherosclerosis, where a linear correlation trend was observed between TSH, on the one hand, and FA and CA intima-media thickness, on the other. For intergroup comparison it's likely that the Pearson coefficient showed an active direct correlation with group II, namely, between TSH, LDLC and CA wall thickness ratios. Thus, the latter is one of the most reliable criteria for comparison between groups (Fig. 3).

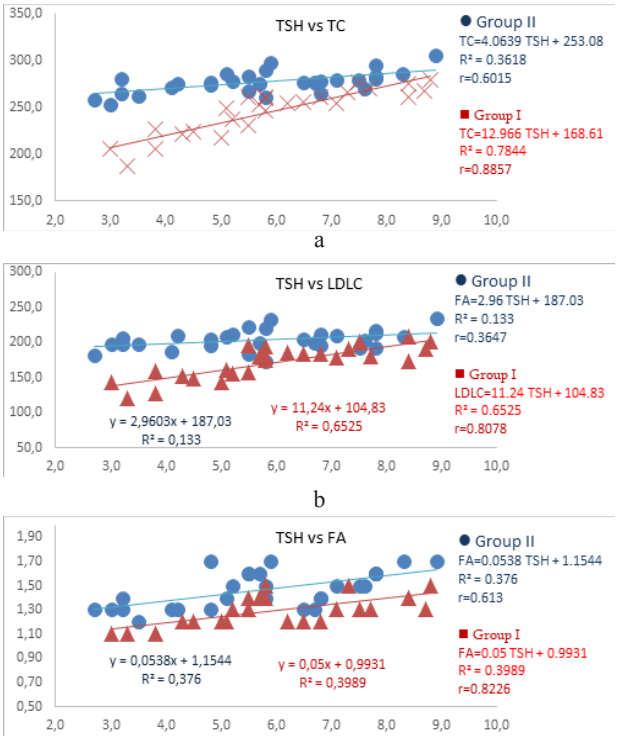

Fig. 3. Pearson correlation (r) between: a - TSH and TC in patients with HT and HT+At (group I and II); b - TSH and LDLC in patients with HT and HT+At (group I and II); c - TSH and FA in patients with HT and HT+At (group I and group II)

We can conclude that TSH, as the main thyroid function regulator, may be determined as principal risk-factor, which independently affects the thyroid morphology as well as carotid and femoral arteries IMT.

Baseline characteristics and Histology of Thyroid parenchyma

In generally, the histopathological diagnosis of HT was based on the presence of diffuse, chronic, inflammatory cells infiltrate, mainly composed of T-lymphocytes and plasma cells and macrophage, organized in germinal centers, also fibrotic areas presents, which did not extend beyond the capsule. The infiltrate had to occur in a normal region of the thyroid gland, as well as the presence of atrophic follicles with numerous Hürthle cells and enlarged thyroid cells, characterized by abundant cytoplasm, which was eosinophilic.

Group I (HT) - results of histopathological research of Hashimoto's thyroiditis causing subclinical hypothyroidism is associated with activity of parallel immunohistochemical reactions, indicating that the thyroid parenchyma is non-homogeneous in terms of parenchyma cell components, as well as molecular biological features. Hashimoto's thyroiditis leading histopathological process is the extensive lymphocytic infiltration of thyroid parenchyma (Fig. 4a), which is accompanied by hypertrophy/hyperplasia of lymphoid follicules and germinal centers, with the abundance of plasma cells and macrophages. In the thyroid parenchyma necrosis areas were not detected.

The high nuclear expression of the protein S100 in HT parenchyma indicates on the dysplasia of the thyroid parenchyma and disorganization of the architecture. Expression of high-intensity S100 protein is associated with Hashimoto's infiltrative foci in the domain between follicles (Fig. 4b).

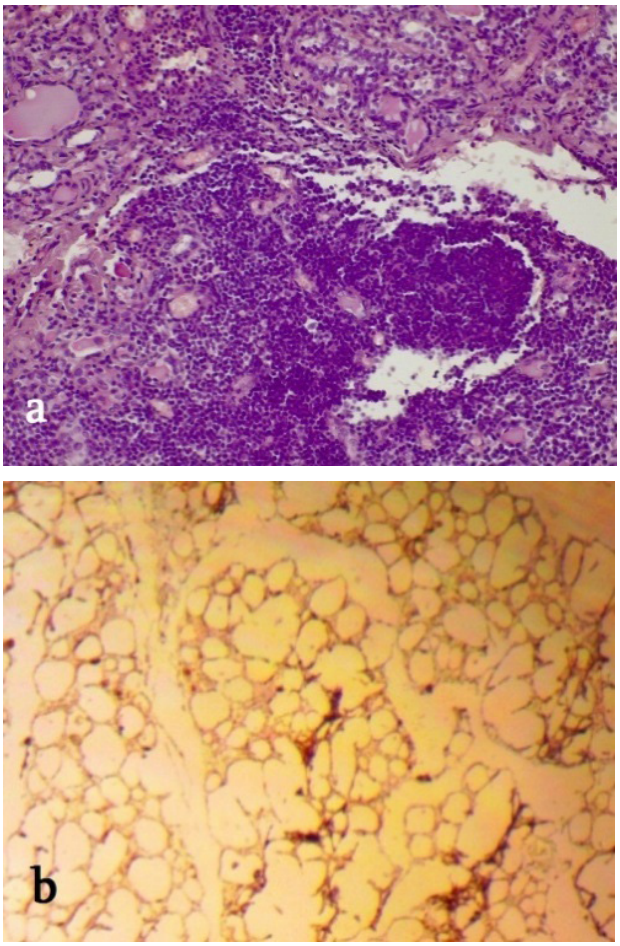

Fig. 4. HT. a. H&E. Atrophic follicles in the thyroid parenchyma, invasive lymphoid follicles with large germination center, X200. b. S100-protein expression is manifested in the domain between follicles in abundant Hürthle cells. Immunoperoxidase reaction, X160

Group II (HT+atherosclerosis) - In the material of the given group marked the typical histological features of Hashimoto's thyroiditis include moderate lymphoplasmacytic infiltration, follicular destruction following with variable degrees of fibrosis (Fig. 5a).

It is significant, that HT with atherosclerosis association characterised by independent line of Hürthle cells and their

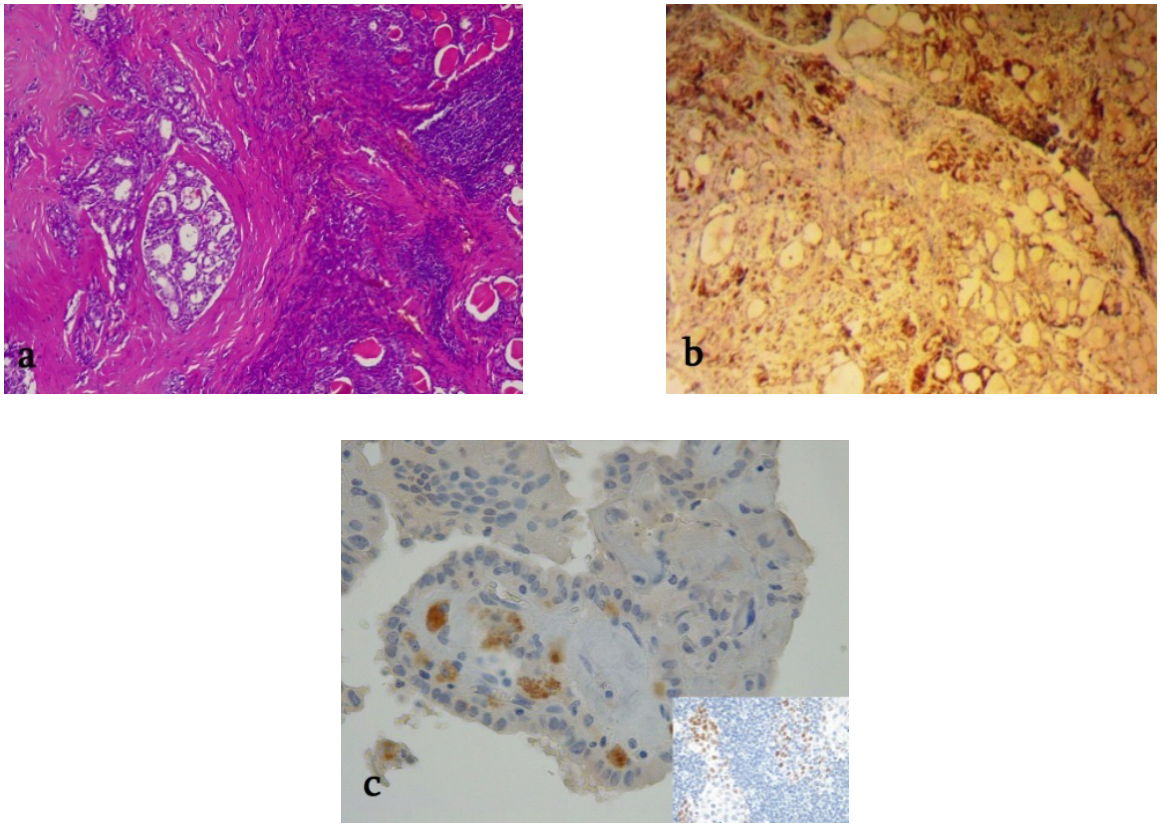

Fig. 5. HT. a. H&E, X100. b. Association of Hürthle cells adenoma and Hashimoto's Thyroiditis. Intense expression of S100 protein on the periphery of the adenoma and in the adenoma capsule, X160. c. Follicular epithelial squamous metaplasia with intense nuclear positive p63 immunostaining, X400; (in rectangle area – control reaction with lymphoid tissue from Palatine Tonsils, X200); Immunoperoxidase reaction

adenoma like hyperplasia, reducing follicular parenchyma, which decrease secretary activity, in fact, hypothyreosis reinforce (Fig. 5b).

In group II (HT+atherosclerosis), new morphological fact develops in some areas of thyroid parenchyma as oxyphilic metaplasia of follicular cells indicating on the severe molecular biological transformation of thyroid parenchyma due to higher low-density lipoprotein cholesterol concentration than in group I (Fig. 5c). Patient from this group also displayed greater CIMT than controls (Fig. 3a, b).

Atherosclerosis and CVD events reduction is very important goal of modern medical society. That is why, beside of the traditional risk factors (dyslipidemia, obesity, hypertension, etc.), scientists attention is focused on the research of the new factors, such as metabolic risk factors and hypothyroidism among them. Taking account the numerous studies about immunological pathogenesis of atherosclerosis [25,26] the importance of Hashimoto thyroiditis influence on CVD events is evident. By 2019 ESC/EAS Guideline [6], next to coronarography, Doppler ultrasound method of carotid and femoral arteries atherosclerotic changes is accepted for confirmation of ACVD.

Based on the above considerations, we examined patients who were operated on due to Hashimoto thyroiditis indications: group I patients HT without atherosclerosis clinical manifestation and group II patients with HT+Atherosclerosis and group III – Atherosclerosis as in control version.

To pick out the importance of atherosclerosis risk factors (dyslipidemia) in the development of atherosclerosis we compared this data with markers of atherosclerosis using carotid and femoral arteries Doppler ultrasonography. This comparison allows immunological factors (anti-TPO) pathologic mechanism influence in patients with HT with atherosclerosis risk-factors. Data of HT+At group's patients vs group's III Atherosclerosis patients accentuate immunological factor (anti-TPO) action on the dyslipidemia that can promote further severity of the atherosclerosis. This point of view is supported by the results of immunohistochemical study of the p63 protein: It was during the combination of atherosclerosis and Hashimoto that foci of p63 expression of squamous epithelial dysplasia were detected (Fig. 5C).

The major risk factors of atherosclerosis – TC, LDLC, TG high and HDLC low levels relation with anti TPO, TSH and FT4 revealed lipid parameters statistically high level in the group II patients but despite high levels of atherogenic lipids, the same status was statistically low in group III (patients with atherosclerosis but without HT) in comparison with group II as well as in group I patients. This fact confirms hypothyreoidism with anti TPO importance in processing of atherosclerosis and HT as atherogenic risk factors significance the correlation under anti-TPO between TSH and TC. LDLC in the I (r =0.89**, 0.81**) and II groups' patients confirmed the immunological status influences in the development of atherosclerosis.

Thus the influence of thyroid hormones on CVD is in conclusive [27]. FT4 levels in middle-aged person are positively associated with At, independently caused cardiovascular risk factors [4,28-31]. In turn, At adversely affected on the lipid cholesterol

and carbohydrates rates metabolism, accelerating hypothyroidism with follicular epithelial meta- and dysplasia manifested in our study by: 1. Hurtle cells activity – adenomatous transformation, 2. follicular cell, oxyphilic metaplasia and focal dysplasia (p63 positivity) [14,17,32]. It's important that in euthyroid individual there was no significant difference between compared date [24, 33].

We suppose, that thyroid hormone plays an important role in the pathogeneses of atherosclerosis and cardiovascular complication through multifunctional physiological effects – such intranuclear genomic and extranuclear nongenomic influences: [26, 34] 1. thyroid hormone acts on the vascular smooth muscles cells, modified endothelial function developing systemic vascular resistance [2,4,33,35,36] and diastolic blood pressure instability [21,33]; 2. Thyroid hormone also reduced LDL and decrease LDL receptor activity [26, 37, 38].

It's well known, that the dyslipidemia and the diastolic hypertension predispose the hypothyroidism in HT and At combination group to accelerate carotid artery IMT. Respectively, our results are in good agreement with this opinion [37, 38].

In the current study we found that free T4 is associated with the severity of atherosclerosis clinical characteristics, but we also found, that TSH and anti-TPO antibody levels are directly and closely linked to the cardiovascular complications (Myocardial infarctions and hypertension).

Furthermore, as discussed above, biomarkers S100 and p63 data results demonstrate negative feedback effects of hypercholesterolemia on the high morphological risk features in Hashimoto parenchyma, which may partially explain the significant trend and pathobiological link of Hashimoto Thyroiditis association with Papillary thyroid carcinoma [13,14,24,25].

Doppler ultrasonography investigation data, revealing CA and FA atherosclerosis and TSH influence, show the presence: 1. FA is most important location for developments of IM complex thickening in HT (group I) and HT+At (group II) confirmed by presence of reliable differences ($p<0.001$) between II and III groups as between the I and III groups patients. 2. CA thickening characterised all three groups' patients, but more expressed in the group II patients. 3. TSH levels clarify the atherogenic quality by presence of correlation between TSH and FA atherosclerosis ($r=0.62$*) as between TSH and CA atherosclerosis ($r=0.6$* in the group I patients). Anti atherogenic HDLC level, statistically reliable, is highest in the group I patients in comparison to II and III groups' patients that can explain absence of clinical manifestation of atherosclerosis despite of the thickening of CA and FA.

Conclusion. Comparative analysis of key phenomena of HT and Atherosclerosis features show that free T4 is associated with the severity of atherosclerosis clinical characteristics, that TSH and anti-TPO antibody levels are directly and closely linked to the cardiovascular complications (Myocardial infarctions and hypertension). Dyslipidemia and the diastolic hypertension accelerate the hypothyroidism in HT and At combination group to predispose carotid artery IMT. Biomarkers S100 and p63 data results demonstrate negative feedback effects of hypercholesterolemia on the high morphological risk features in Hashimoto parenchyma, which may be partially explain the significant trend and pathobiological link of Hashimoto Thyroiditis with Papillary thyroid carcinoma.

Data, presented in the study, will serve as a reference for further investigation.

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SUMMARY

CORRELATION OF THYROID AUTOIMMUNITY WITH ATHEROSCLEROSIS EVALUATION IN HASHIMOTO'S THYROIDITIS

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The relationship between subclinical hypothyroidism (SH) and Atherosclerotic (At) cardiovascular diseases (CVD) has been one of the most popular topics but causal connection between Hashimoto thyroiditis (HT), lipid profile and follicular

epithelial molecular biology is controversial. We investigated 3 groups of patients (group I – HT, group II - HT+At, group III - At). All laboratory tests for thyroid function and lipid profile detection were used according to international guideline recommendations, coronary and femoral arteries intima-media thickness (IMT) were tested by high-resolution ultrasonography, thyroid gland histology and immunohistochemistry carried out by p63 and S100 protein expression control. The statistical analysis was performed using Microsoft Excel 7.0, SPSS-20 version, Mann–Whitney U–test and Pearson's correlation. Comparisons between groups and factors were made using Multiple Linear Regression model. With the results obtained, dyslipidemia and the diastolic hypertension accelerate the hypothyroidism in HT+At group to predispose carotid and femoral arteries IMT. TSH and anti-TPO antibody levels are directly linked to the cardiovascular complications. Biomarkers S100 and p63 data show negative feedback effects of hypercholesterolemia on the high morphological risk features in Hashimoto parenchyma, which may partially explain the significant trend and pathobiological link of HT with Papillary thyroid carcinoma.

Keywords: Hashimoto Thyroiditis; Atherosclerosis; Thyroid; p63, S100 immunohistochemistry; Carotid, Femoral IMT.

РЕЗЮМЕ

КОРРЕЛЯЦИЯ МЕЖДУ АУТОИММУННЫМ ТИРОИДИТОМ И РАЗВИТИЕМ АТЕРОСКЛЕРОЗА ПРИ ТИРОИДИТЕ ХАШИМОТО

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Изучение взаимосвязи между субклиническим гипотиреозом и атеросклерозом сердечно-сосудистой системы (At) представляется актуальным, однако причинно-следственная корреляция между тироидитом Хашимото (HT), липидным профилем и молекулярной биологией фолликулярного эпителия щитовидной железы по сей день остается малоизученной.

Исследованы 3 группы пациентов: I группа – HT, II группа – HT+At, III группа – At. Использованы лабораторные тесты с целью определения функции щитовидной железы и липидного профиля, согласно указаниям международных гайдлайнов. Толщину интимы-медии сонных и бедренных артерий (IMT) оценивали высокоразрешенной ультрасонографией. Материал исследовали гистологическими и иммуногистохимическими методами: H&E, S100 протеин и p63.

Статистический анализ проводили по версии Microsoft Excel 7.0, SPSS 20, Mann-Whitney. Использовали U-тест и коэффициент корреляции Пирсона. Сравнительный межгрупповой анализ проводили методом линейной регрессии.

Согласно полученным результатам, дислипидемия и диастолическая гипертензия способствуют прогрессии гипотиреоза в группе HT+At; уровень TSH и anti-TPO антител находится в прямой зависимости от осложнений сердечно-сосудистых заболеваний. S100 и p63 биомаркеры указывают на обратный отрицательный эффект гиперхолестеринемии, на показатели высокого морфологического риска в паренхиме Хашимото, что частично объясняет тенденцию HT и патобиологическую связь с папиллярной карциномой.

რეზიუმე

ფარისებრი ჯირკვლის აუტოიმუნურობის კორელაცია ათეროსკლეროზის განვითარებასთან ჰაშიმოტოს თიროიდიტის დროს

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სუბკლინიკური ჰიპოთირეოზისა და გულ-სისხლძარღვთა ათეროსკლეროზული (At) დაავადებების ურთიერთკავშირი ერთ-ერთი აქტუალური თემაა, მაგრამ სადავოა ჰაშიმოტოს თირეოიდიტს (HT), ლიპიდურ პროფილსა და ფოლიკულური ეპითელიუმის მოლეკულურ ბიოლოგიას შორის მიზეზობრივი კავშირი. გამოკვლეულია პაციენტების 3 ჯგუფი (I ჯგუფი - HT, II ჯგუფი - HT+At,III ჯგუფი - At). ფარისებრი ჯირკვლის ფუნქციისა და ლიპიდური პროფილის განსაზღვრისთვის გამოყენებული იყო ლაბორატორიული ტესტები საერთაშორისო გაიდლაინების მითითებების შესაბამისად, კორონარული და ბარძაყის არტერიების ინტიმა-მედიის სისქე (IMT) შემოწმდა მაღალი რეზოლუციის ულტრასონოგრაფიით, მასალა შესწავლილია კლასიკური ჰისტოლოგიური და იმუნოჰისტოქიმიური კვლევის მეთოდებით: H&E, S100 ცილა და p63-ის იმუნური პროფილის გათვალისწინებით. სტატისტიკური ანალიზი ჩატარდა Microsoft Excel 7.0, SPSS-20 ვერსიის, Mann-Whitney U-ტესტისა და პირსონის კორელაციის გამოყენებით. ჯგუფებსა და ფაქტორებს შორის შედარება განხორციელდა ხაზოვანი რეგრესიის მოდელის გამოყენებით. მიღებული შედეგების მიხედვით, დისლიპიდემია და დიასტოლური ჰიპერტენზია აჩქარებს ჰიპოთირეოზის განვითარებას HT+At ჯგუფში. TSH და anti-TPO ანტისხეულების დონე პირდაპირკავშირშია გულ-სისხლძარღვთა დაავადებების გართულებასთან. S100 და p63 ბიომარკერების მონაცემები აჩვენებს ჰიპერქოლესტერინემიის უარყოფით გავლენას ჰაშიმოტოს პარენქიმაში მაღალი მორფოლოგიური რისკის მახასიათებლებზე, რითაც ნაწილობრივ შეიძლება აიხსნას ჰაშიმოტოს მნიშვნელოვანი ტენდენცია და პათობიოლოგიური კავშირი ფარისებრი ჯირკვლის პაპილურ კარცინომასთან.

PHENOTYPIC CHARACTERISTICS OF RELAPSED LEIOMYOMA AND SMOOTH MUSCLE TUMORS OF UNCERTAIN MALIGNANCY POTENTIAL IN REPRODUCTIVE WOMEN

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Uterine leiomyoma, represents the most commons pelvic tumor in females. The incidence of leiomyoma represents 20% and 40% in less than 30 and 40 years old females respectively [1]. Leiomyomas are benign monoclonal tumors, which arise from the smooth muscle cells of the myometrium. There are numerous histological subtypes of uterine leiomyoma, including classic, cellular, bizzare/atypical and smooth muscle tumors of uncertain malignancy potential (STUMP) and others [2]. Leiomyosarcomas represent the malignant counterparts of leiomyoma. Although extremely rare, leiomyoma can transform into leiomyosarcoma [3].

In recent years, laparoscopic myomectomy developed as a treatment of choice for leiomyoma, as it represents the less invasive procedure [4]. However, it has been noted that the relapse of leiomyoma can occur after laparoscopic myomectomy [5]. The rate of leiomyoma relapse represents 11.7%, 36.1%, 52.9% and 84.4% after 1, 3, 5 and eight years from laparoscopic myomectomy respectively [6]. Therefore, the investigation of molecular markers, indicating risk of relapse after laparoscopic myomectomy is of high importance.

Smooth muscle tumors with uncertain malignancy potential (STUMP) represent the group of smooth muscle tumors, which cannot be diagnosed surely as benign or malignant [7]. Therefore, the clinical management of this entity is complicated. Mostly, they show relatively non-aggressive behaviour, compared to leiomyosarcomas and survival rates are also relatively higher. However, in 8.7% to 11% of cases the relapse can develop[7]. Therefore, the investigation of molecular markers which indicate the relatively benign or aggressive behaviour of this tumors is of high interest.

In our current study, we investigated the molecular phenotype of different types of leiomyomas, including STUMP, in hysterectomy and laparoscopic myomectomy specimens from patients in reproductive and menopausal age.

Material and methods. *Tissue samples.* Formalin fixed and paraffin embedded tissue material was retrieved from the Research, Diagnostic and Teaching Laboratory of Tbilisi State Medical University, Georgia. Study included altogether 237 tissue specimens distributed in two major groups: group I specimens received by histerectomy (n=155) and specimens received by laparoscopic miomectomy (n=82). Specimens were further subdivided into following categories: from patients in reproductive age and from patients in menopause. Group I included n=102 specimens from reproductive age patients and n=53 specimens from menopausal patients. Group II included n=52 specimens from reproductive age patients and n=30 specimens from menopausal patients. Group I included following histological subtypes: classic leiomyoma (n=69), cellular leiomyoma (n=15), bizzare/atypical leiomyoma (n=22), smooth muscle tumors with uncertain malignancy potential (STUMP) (n=17), leiomyosarcoma (n=12) and control group of normal myometrium (n=20); Group II included following histological subtypes: classic leiomyoma (n=35), bizzare/atypical leiomyoma (n=18) and STUMP (n=29). Cases in group II were further subdivided into relapsed cases and control group.

Immunohistochemistry. 4μ FFPE tissue sections were deparaffinized in xylene, rehydrated by using serial dilutions of ethanol (96%, 80%, 70%) and heat mediated antigen retrieval has been performed. Ready to use antibodies against the following antigens were used: Ki67 (K2), cyclin D1 (polyclonal), Bcl2 (bd-2/100/05) cleaved Cas3, ER (6f11) and PR (16) (Novocastra). Staining and visualisation has been performed using Bond polymer refine detection system. The number of positive cells were counted in 20HPF and the following indexes were made: proliferation index – based on Ki67 and cyclin D1 labelling, apoptotic index – based on Bcl2 and Cas3 labelling, ER index and PR index. Proliferation and apoptosis index 0-10% was considered as low and >10% was considered as high. The ER and PR index 0-10% was considered as low, the index 10-50% was considered as moderate and the index >50% was considered as high.

Comparisons between groups were made using Mann-Whitney U and Kruskall-wallis test and correlations were assessed using Spearman's rank correlation. P values <0.05 were considered as significant. All statistical tests were performed using SPSS software V19.00.

Results and discussion. The results of Ki67, Cyclin D1, BCL2, Cas3, ER and PR analysis in myomectomy specimens showed the following results: in normal myometrium mean Ki67 expression was 1.7 in reproductive age and 0.4 in menopause; mean Cyclin D1 expression level was 1.2±0.3 in reproductive age and 0.2±0.05 in menopause; mean Bcl2 expression level was 0.9±0.1 in reproductive age and 2.1±0.7 in menopause; mean Cas3 expression level was 0.7±0.3 in reproductive age and 1.8 in menopause; mean ER expression level was 80 in reproductive age and 40 in menopause and mean PR expression level was 89.6±6.9 in reproductive age and 42±7.1 in menopause. In classic leiomyoma, the mean Ki67 expression was 3±1.1 in reproductive age and 1.2±0.8 in menopause; mean Cyclin D1 expression level was 2.4±1.2 in reproductive age and 0.9±0.5 in menopause; mean Bcl2 expression level was 6.7±2.1 in reproductive age and 8.3±3.2 in menopause; mean Cas3 expression level was 5.4±2.4 in reproductive age and 7.9±3.3 in menopause; mean ER expression level was 70.2±10.3 in reproductive age and 34.3±5.6 in menopause and mean PR expression level was 75.7±9.7 in reproductive age and 36.3±6.9 in menopause. In cellular leiomyoma the mean Ki67 expression was 8.9±3.6 in reproductive age and 3.4±1.4 in menopause; mean Cyclin D1 expression level was 7.6±3.4 in reproductive age and 3.1±1.1 in menopause; mean Bcl2 expression level was 9.6±3.9 in reproductive age and 10.9±4.1 in menopause; mean Cas3 expression level was 8.5±2.9 in reproductive age and 9.9±3.2 in menopause; mean ER expression level was 60.5±9.4 in reproductive age and 29.1±4.8 in menopause and mean PR expression level was 63.2±7.1 in reproductive age and 30.2±5.5 in menopause. In bizzare/atypical leiomyoma the mean Ki67 expression was 12.5±3.8 in reproductive age and 4.7±1.1 in menopause; mean Cyclin D1 expression level was 10.5±4.4 in reproductive age and 3.5±0.9 in menopause; mean Bcl2 expression level was 13.3±3.2 in reproductive age and 15.7±4.5

Table 1. Distribution of Ki67, Cyclin D1, Bcl2, Cas3, ER and PR in myomectomy tissue specimens

Normal Miometrium	Repr. Ag.	10	1,7	1,2	81,3	0,7	80	89,6
	Menop. Ag.	10	0,4	0,2	89,2	1,8	40	42
Classic LM	Repr. Ag.	45	3	2,4	67,6	5,4	70,2	75,7
	Menop. Ag.	24	1,2	0,9	71,2	7,9	34,3	36,3
Cellular LM	Repr. Ag.	10	8,9	7,6	59,7	8,5	60,5	63,4
	Menop. Ag.	5	3,4	3,1	62,3	9,9	29,1	30,2
Bizzare/Atypical LM	Repr. Ag.	14	12,5	10,5	13,3	11,2	50,6	52,1
	Menop. Ag.	8	4,7	3,5	15,7	13,8	23,8	23,9
STUMP	Repr. Ag.	13	20,8	18,6	15,6	7,6	43,9	46,8
	Menop. Ag.	4	6,3	5,2	17,3	9,3	17,6	18,7
LMS	Repr. Ag.	10	42,1	40,2	3,8	2,9	35,7	40,7
	Menop. Ag.	2	10,9	8,7	4,2	3,7	12,4	13,5

in menopause; mean Cas3 expression level was 11.2±2.2 in reproductive age and 13.8±3.6 in menopause; mean ER expression level was 50.6±8.4 in reproductive age and 23.8±6.7 in menopause and mean PR expression level was 52.1±7.1 in reproductive age and 23.9±3.7 in menopause. In STUMP the mean Ki67 expression was 20.8±11.4 in reproductive age and 6.3±1.5 in menopause; mean Cyclin D1 expression level was 18.6±10.8 in reproductive age and 5.2±1.3 in menopause; mean Bcl2 expression level was 8.7±2.1 in reproductive age and 10.6±3.5 in menopause; mean Cas3 expression level was 7.6±2.5 in reproductive age and 9.3±2.9 in menopause; mean ER expression level was 43.9±5.6 in reproductive age and 17.6±4.8 in menopause and mean PR expression level was 46.8 in reproductive age and 18.7±5.6 in menopause.

The results of Ki67, Cyclin D1, BCL2, Cas3, ER and PR analysis in laparoscopic surgical specimens showed the following results: in classic leiomyoma the mean Ki67 labelling index was 6.9±2.2 in relapsed and 3.1±1.3 in control specimens from patients in reproductive age. In patients with menopause the mean Ki67 labelling index was 3.7±1.4 and 1.4±0.6 in relapsed and control cases respectively. Mean cyclin D1 labelling index was 5.3±2.4 in relapsed and 2.7±1.8 in control specimens from reproductive age patients. In patients with menopause mean cyclin D1 labelling index was 2.1±0.4 and 1±0.3 in relapsed and control groups respectively. Mean Bcl2 labelling index was 3.2±0.9 in relapsed and 5.9±1.7 in control cases from reproductive age patients. In patients with menopause mean Bcl2 labelling index was 3.9±1.9 and 8.8±2.7 in relapsed and in control cases respectively. Mean Cas3 labelling index was 2.9±0.8 in relapsed and 5.1±1.9 in control cases from patients in reproductive age and 3.1±0.8 and 7.2±2.9 in relapsed and control group respectively, in patients with menopause. Mean ER positivity was 85.8±9.6 in relapsed and 79.6±7.9 in control group in reproductive age patients, whilst it was 48.2±8.2 and 42.9±6.3 in menopausal patients in relapsed and control groups respectively. Mean PR positivity was 90.6±9.1 in relapsed group and 85.7±7.8 in control group in reproductive age patients, whilst it was 50.7±5.5 and 46.2±4.9 in menopausal patients in relapsed and control groups respectively; In bizzare/atypical leiomyoma the mean Ki67 labelling index was 26±3.9 in relapsed and 12.3±2.7 in control specimens from patients in reproductive age. In patients with menopause the mean Ki67 labelling index was 11.4±3.3 and 4.9±1.8 in relapsed and control cases respectively. Mean cyclin D1 labelling index was 24.5±4.6 in relapsed and 11.2±3.7 in control specimens from reproductive age patients. In patients with menopause mean cyclin D1 labelling index was 10.6±2.9 and 3.8±1.1 in relapsed and control groups respectively. Mean Bcl2 labelling index was 5.7±2.1 in relapsed and 13.9±4.1 in control cases from reproductive age patients. In patients with menopause mean Bcl2 labelling index was 7.9±3.9 and 16.2±4.5 in relapsed and in control cases respectively. Mean Cas3 labelling index was 4.9±2.1 in relapsed and 12.3±3.8 in control cases from patients in reproductive age and 6.8±2.3 and 14.1±3.5 in relapsed and control group respectively, in patients with menopause. Mean ER positivity was 65.6±10.3 in relapsed and 47.5±8.5 in control group in reproductive age patients, whilst it was 40.1±5.5 and 30.2±6.3 in menopausal patients in relapsed and control groups respectively. Mean PR positivity was 68.9±9.2 in relapsed group and 50.3±8.7 in control group in reproductive age patients, whilst it was 47.2±4.3 and 35.1±2.3 in menopausal patients in relapsed and control groups respectively; In STUMP the mean Ki67 labelling index was 61.4±8.6 in relapsed and 21.2±3.3 in control specimens from patients in reproductive age. In patients with menopause the mean Ki67 labelling index was 21.8±6.9 and 7.3±2.1 in relapsed and control cases respectively. Mean cyclin D1 labelling index was 52.7±7.6 in relapsed and 19.1±4.7 in control specimens from reproductive age patients. In patients with menopause mean cyclin D1 labelling index was 18.6±3.3 and 5.7±2.2 in relapsed and control groups respectively. Mean Bcl2 labelling index was 3.3±0.9 in relapsed and 9.2±3.3 in control cases from reproductive age patients. In patients with menopause mean Bcl2 labelling index was 4.8±1.8 and 11.3±3.4 in relapsed and in control cases respectively. Mean Cas3 labelling index was 2.6±0.9 in relapsed and 8.1±2.6 in control cases from patients in reproductive age and 3.7±1.3 and 9.8±3.3 in relapsed and control group respectively, in patients with menopause. Mean ER positivity was 53.7±9.4 in relapsed and 42.2±7.5 in control group in reproductive age patients, whilst it was 30.9±6.9 and 20.8±5.8 in menopausal patients in relapsed and control groups respectively. Mean PR positivity was 57.1±8.8 in relapsed group and 44.3±5.4 in control group in reproductive age patients, whilst it was 36.3±6.7 and 23.6±7.9 in menopausal patients in relapsed and control groups respectively.

Table 2. Distribution of Ki67, Cyclin D1, Bcl2, Cas3 in laparoscopy tissue specimens

		Rel.	Cont.	Rel.	Cont.	Rel.	Cont.	Rel.	Cont.	Rel.	Cont.
Classic LM	Repr. Ag.	13	9	6,9	3,1	5,3	2,7	69,3	65,2	2,9	5,1
	Menopause	7	6	3,7	1,4	2,1	1	71,6	68,7	3,1	7,2
Bizzare/ Atypical LM	Repr. Ag.	6	5	26	12,3	24,5	11,2	17,2	14,3	4,9	12,3
	Menopause	4	3	11,2	4,9	10,6	3,8	18,1	16,2	6,8	14,1
STUMP	Repr. Ag.	12	7	61,4	21,2	52,7	19,1	15,1	13,4	2,6	8,1
	Menopause	6	4	21,8	7,3	18,6	5,7	15,8	12,9	3,7	9,8

Table 3. Distribution of ER and PR in laparoscopy tissue specimens

				ER		PR	
		Rel.	Cont.	Rel.	Cont.	Rel.	Cont.
Classic LM	Repr. Ag.	13	9	85,8	79,6	90,6	85,7
	Menopause	7	6	48,2	42,9	50,7	46,2
Bizzare/Atypical LM	Repr. Ag.	6	5	65,6	47,5	68,9	50,3
	Menopause	4	3	40,1	30,2	47,2	35,1
STUMP	Repr. Ag.	12	7	53,7	42,2	57,1	44,3
	Menopause	6	4	30,9	20,8	36,3	23,6

Further investigation results of proliferative and apoptotic markers in both groups showed the following results: in group I (myomectomy specimens) in reproductive age patients cellular leiomyomas, bizzare/atypical leiomyoma and leiomyosarcomas were divided into two major groups based on the expression of Ki67, cyclin D1, Bcl2 and Cas3. Particularly, in cellular leiomyomas low Ki67 labelling index (<=10) were present in 6/10 cases (60%), whilst high Ki67 labelling index (>10) was present in 4/10 (40%) cases. Low cyclin D1 labelling index was also presented in 6/10 (60%) cases and high cyclin D1 labelling index was presented in 4/10 (40%) cases. Low Bcl2 labelling index was presented in 7/10 (70%) cases and high Bcl2 labelling index was presented in 3/10 (30%) cases, similar to Cas3 labelling index, which was also presented as low in 7/10 (70%) and high in 3/10 (30%) cases. In bizzare/atypical leiomyomas low Ki67 index was presented in 8/14 (57.1%) cases and high Ki67 labelling index was presented in 6/14 (42.9%) cases similar to cyclin D1, which was also presented as low in 8/14 (57.1%) and as high in 6/14 (42.9%) cases. Low Bcl2 and Cas3 was presented in 9/14 (64.3%) cases and high Bcl2 and Cas3 was presented in 5/14 (35.7%) cases. In leiomyosarcoma low Ki67 and cyclin D1 index was presented in 2/10 (20%) cases and high Ki67 and cyclin D1 was presented in 8/10 (80%) cases. Low Bcl2 and Cas3 was presented in 9/10 (90%) cases, whilst high Bcl2 and Cas3 was presented in 1/10 (10%) case.

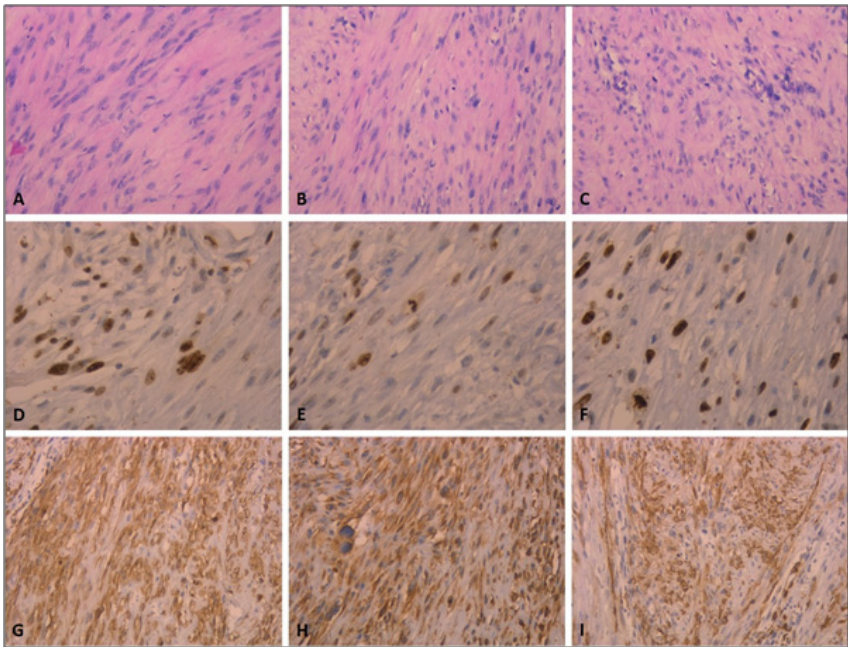
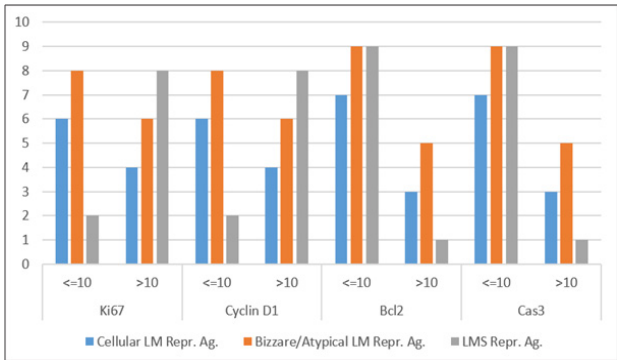
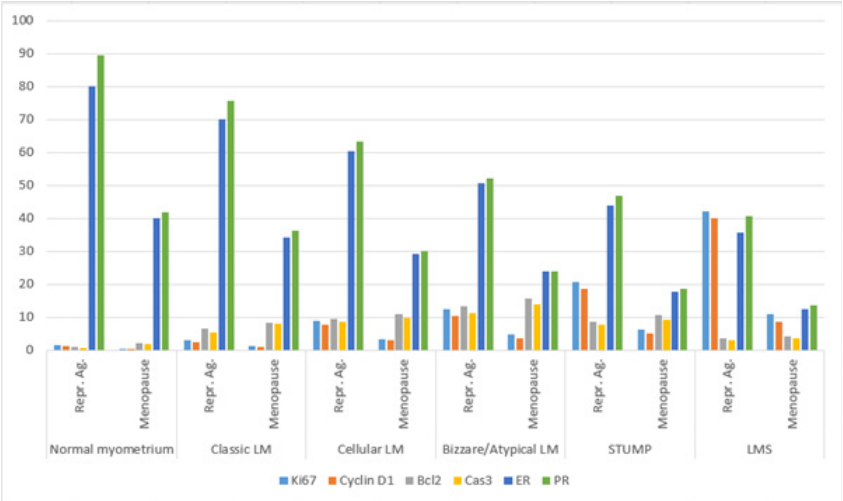


Fig. 1. A. Classic leiomyoma, H&E, x200 B. Bizzare/atypical leiomyoma, H&E, x200 C. STUMP, H&E, x200, D. Ki67 in bizzare/atypical leiomyoma, IHC, x200, E. Ki67 in STUMP, IHC, x200, F. Ki67 in leiomyosarcoma, IHC, x200, G. Bcl2 in classic leiomyoma, IHC, x200, G. Bcl2 in STUMP, IHC, x200, I. Bcl2 in , IHC, x200

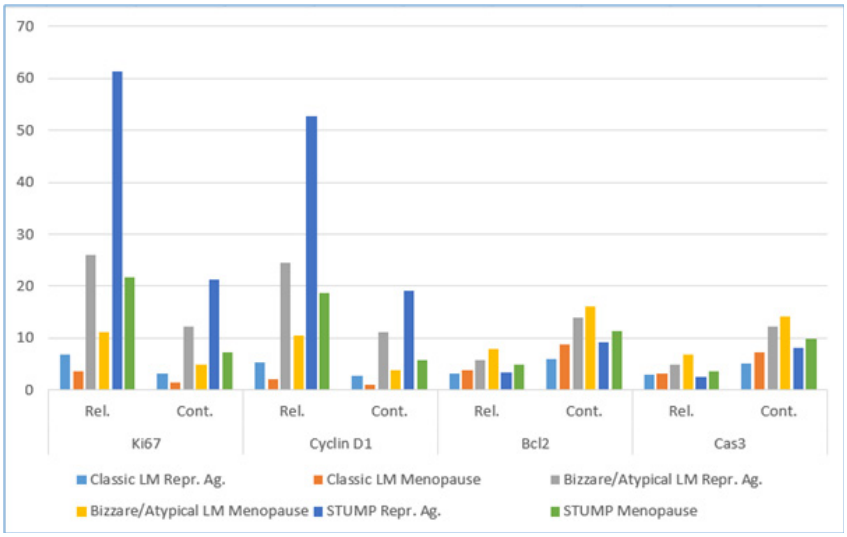


Graph 1. Distribution of cellular LM, bizzare/atypical LM and LMS cases in high and low proliferation and apoptotic groups in myomectomy specimens of reproductive women

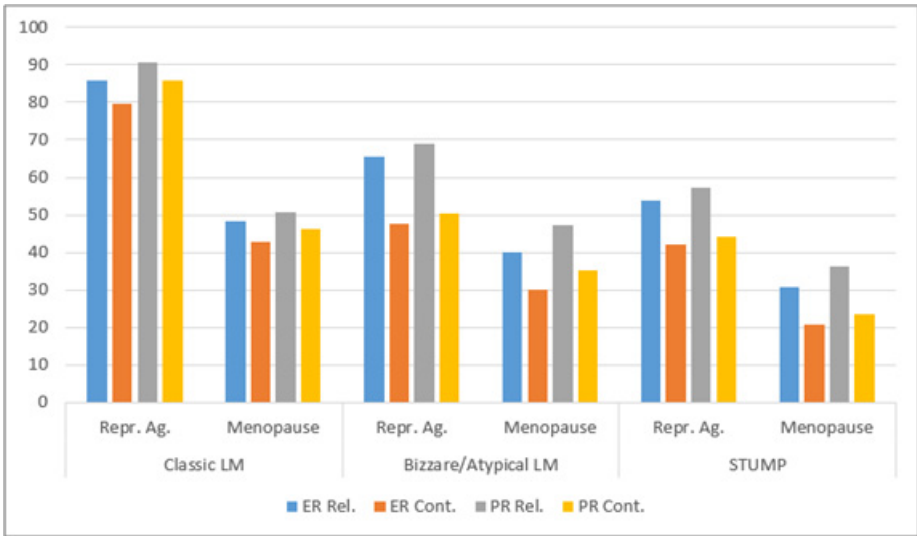
In laparoscopic surgical specimens, similar trend has been seen in groups with classic leiomyoma and bizzare/atypical leiomyomas. In classic leiomyoma in control group, 6/9 (66.7%) cases were characterised with low and 3/9 (33.3%) cases were characterised with high Ki67 and cyclin D1 labelling index. In relapsed group, 5/13 (38.5%) cases were characterised with low and 8/13 (61.5%) cases were characterised with high Ki67 and cyclin D1 labelling index. On the other hand, in control group 7/9 (77.8%) cases were characterised with low Bcl2 and Cas3 labelling index, whilst 2/9 (22.2%) cases were characterised with high Bcl2 and Cas3 labelling index. In relapsed group, 9/13 (69.2%) cases were characterised with low Bcl2 and Cas3 labelling index and 4/13 (30.8%) cases were characterised with high Bcl2 and Cas3 labelling index. In bizzare/atypical leiomyomas in control group 2/5 (40%) cases were characterised with low Ki67 and Cyclin D1 labelling index and 3/5 (60%) cases were characterised with high Ki67 and cyclin D1 labelling index. In relapsed group, 1/6 (16.6%) case were characterised with low Ki67 and cyclin D1 labelling index and 5/6 (83.4) cases were characterised with high Ki67 and cyclin D1 labelling index. With regard to apoptotic index, in control group 3/5 (60%) cases were characterised with low Bcl2 and Cas3 index and 2/5 (40%) cases were characterised with high Bcl2 and Cas3 index. In relapsed group, 3/6 (50%) cases were characterised with low Bcl2 and Cas3 index and 3/6 (50%) cases were characterised with high Bcl2 and Cas3 index.



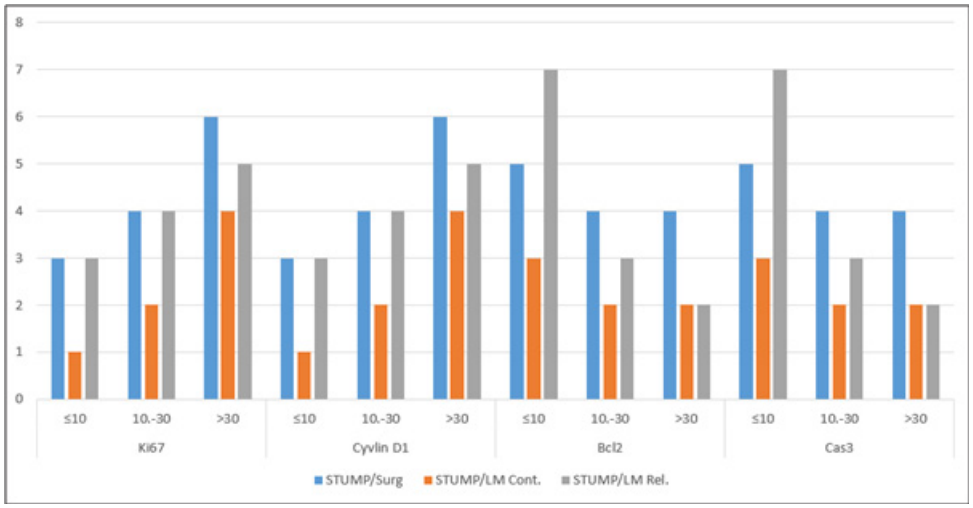
Graph 2. Distribution of cellular LM and bizzare/atypical LM cases in high and low proliferation and apoptotic groups in laparoscopic specimens of reproductive women. LM, leiomyoma, Repr.Ag., reproductive age



Graph 3.The comparative analysis of Ki67, cyclin D1, Bcl2 and Cas3 in laparoscopic myomectomy specimens. LM, leiomyoma, Repr. Ag. reproductive age.



Graph 4. Comparative analysis of ER and PR in laparoscopic surgical specimens. LM, leiomyoma, Cont., control specimens, Rel., relapsed specimens



Graph 5. Distribution of STUMP cases in different proliferative and apoptotic groups, Surg., surgical specimen, LM, laparoscopic myomectomy, Rel., relapsed cases

In cases of STUMP in both group I and group II three groups were identifiable based on proliferation and apoptotic index. Particularly in group I 3/13 (23%) cases were characterised with low Ki67 and cyclin D1 labelling index (<=10), 4/13 (30.8%) cases were characterised with moderate Ki67 and cyclin D1 labelling index (10-30) and 6/13 (46.2%) cases were characterised with high Ki67 and cyclin D1 labelling index (>30). In addition, 5/13 (38.4%) cases were characterised with low Bcl2 and Cas3 labelling index, 4/13 (30.8%) cases were characterised with moderate Bcl2 and Cas3 labelling index and 4/13 (30.8%) cases were characterised with high Bcl2 and Cas3 labelling index. In group II, in control specimens 1/7 (14.3%) cases were characterised with low proliferative index, 2/7 (28.6%) cases were characterised with moderate proliferative index and 4/7 (57.1%) cases were characterised with high proliferative index. On the opposite side, 3/7 (42.9%) cases were characterised with low Bcl2 and Cas3 index, 2/7 (28.6%) cases were characterised with moderate Bcl2 and Cas3 index and 2/7 (28.6%) cases were characterised with high Bcl2 and Cas3 index. In relapsed group 3/12 (25%) cases were characterised with low proliferation index, 4/12 (20%) cases were characterised with moderate proliferation index and 5/12 (55%) cases were characterised with high proliferative index. In addition, 7/12 (58.3%) cases were characterised with low apoptotic index, 3/12 (25%) cases were characterised with moderate apoptotic index and 2/12 (16.7%) cases were characterised with high apoptotic index.

Comparative analysis of studied markers in group I indicated that maximal expression of ER and PR is seen in control group (normal myometrium) and it gradually decreased in leiomyomas, reaching the minimal expression levels in leiomyosarcomas. Ki67 and Cyclin D1 labelling index is higher in all histological subgroups of reproductive age patients, compared to specimens from patients with menopause, whilst the opposite has been seen in cases of Bcl2 and Cas3 expression, which is higher in all histological groups of menopausal patients compared to reproductive patients. Higher expression of proliferation markers was seen in cases with bizzare/atypical leiomioma, STUMP and leiomyosarcoma, compared to classic and cellular leiomyomas. On the opposite the lowest expression apoptotic markers have been seen in cases with bizzare/atypical leiomioma, STUMP and leiomyosarcoma compared to classic and cellular leiomyomas.

In reproductive age patients lowest Ki67 and Cyclin D1 labelling index has been seen in normal myometrium, followed by classic leiomyomas. The expression of Ki67 and Cyclin D1 is almost three times higher in cellular leiomyoma compared to classic leiomyoma and four times higher in bizzare/atypical leiomyoma compared to classic leiomyoma. Whilst in STUMP and leiomyosarcoma the expression of Ki67 and Cyclin D1 is seven times and 14 times higher, respectively. With regard to apoptotic index, the highest apoptotic index has been seen in bizzare/atipycal leiomioma and lowest apoptotic index has been seen in normal myometrium, followed by leiomyosarcoma. Highest ER and PR expression has been seen in normal myometrium and lowest ER and PR expression has been seen in leiomyosarcomas.

Comparative analysis of proliferation and apoptotic proteins in laparoscopic surgical specimens showed that in all histological subtypes of leiomyomas proliferation markers Ki67 and cyclin D1 are expressed at nearly twice as much higher levels in relapsed group, compared to control group in both reproductive and menopausal age women. On the other hand, in the apoptotic markers Bcl2 and Cas3 are expressed almost twice as less levels compared to control group in both reproductive and menopausal patients.

The comparative analysis of ER and PR in laparoscopic surgery group indicated that in all histological subtypes, ER expression was much higher in relapsed group compared to control group. Whilst the progesterone showed the opposite trend.

The analysis of STUMP cases showed that three groups these histopathological entity is identifiable based on proliferation and apoptotic indexes. It has been shown that the majority of STUMP cases which were relapsed, belong to the high proliferative and low apoptotic groups. Whilst in control group or in surgical specimens STUMP cases are relatively equally distributed in different proliferative and apoptotic groups.

It is known that sex steroid hormone oestrogen plays and important role in the pathogenesis of leiomyoma [8]clinical, and experimental evidence. Estradiol and progesterone induce mature leiomyoma cells to release mitogenic stimuli to adjacent immature cells, thereby providing uterine leiomyoma with undifferentiated cells that are likely to support tumor growth. Progesterone action is required for the complete development and proliferation of leiomyoma cells, while estradiol predominantly increases tissue sensitivity to progesterone by increasing the availability of progesterone receptors (PRs. Oestrogen is involved in the upregulation of several genes which cause the leiomyoma formation [8]clinical, and experimental evidence. Estradiol and progesterone induce mature leiomyoma cells to release mitogenic stimuli to adjacent immature cells, thereby providing uterine leiomyoma with undifferentiated cells that are likely to support tumor growth. Progesterone action is required for the complete development and proliferation of leiomyoma cells, while estradiol predominantly increases tissue sensitivity to progesterone by increasing the availability of progesterone receptors (PRs, including growth factors, collagens and oestrogen and progesterone receptors [8]clinical, and experimental evidence. Estradiol and progesterone induce mature leiomyoma cells to release mitogenic stimuli to adjacent immature cells, thereby providing uterine leiomyoma with undifferentiated cells that are likely to support tumor growth. Progesterone action is required for the complete development and proliferation of leiomyoma cells, while estradiol predominantly increases tissue sensitivity to progesterone by increasing the availability of progesterone receptors (PRs. In our study we showed that the expression of ER is significantly higher in laparoscopic myomectomy specimens after relapse, compared to control group or hysterectomy specimens. To the best of our knowledge we are first who demonstrated such a finding. With regard to cell proliferation and apoptotic markers, it is indicated that the balance between these two plays an important role in the development of virtually all types of tumors, including leiomyomas [9]. In our study we demonstrated that the balance between proliferation and apoptotic markers is markedly altered in relapsed leiomyomas. Particularly the expression of cell proliferation markers, particularly Ki67 and cyclin D1 is significantly higher in relapsed cases compared to control group. Whilst the expression of apoptotic markers Bcl2 and Cas3 is significantly decreased. In addition, there is the possibility to divide STUMP cases into three molecular subtypes, based on proliferation and apoptotic indexes. Particularly, these groups include cases with low proliferation and high apoptotic potential, cases with moderate proliferation and apoptotic potential and cases with high proliferation and low apoptotic potential. Later is more similar to leiomyosarcomas, whilst first group is more similar to classic leiomyomas. We are sure, that it is the first demonstration of such a finding.

Conclusions

ER expression is markedly higher in relapsed leiomyomas, compared to control group. Whilst PR shows the opposite trend. This finding can be used as a potential marker for leiomyoma relapse after laparoscopic myomectomy.

The relapsed leiomyomas after laparoscopic myomectomy are characterised with high proliferation and low apoptotic potential, which can also be used as a potential marker for leiomyoma relapse after laparoscopic myomectomy.

STUMP represents the heterogeneous group of smooth muscle tumors, with three different molecular subtype. Particularly, cases with with low proliferation and high apoptotic potential, resembling more to classic leiomyomas, cases with moderate proliferation and apoptotic potential and cases with high proliferation and low apoptotic potential, resembling more to leiomyosarcomas. This finding should be considered in clinical management of these tumors.

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SUMMARY

PHENOTYPIC CHARACTERISTICS OF RELAPSED LEIOMYOMA AND SMOOTH MUSCLE TUMORS OF UNCERTAIN MALIGNANCY POTENTIAL IN REPRODUCTIVE WOMEN

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Uterine leiomyoma represents the most common pelvic tumor in females, including numerous histological subtypes, from which smooth muscle tumors of uncertain malignancy potential (STUMP) represents the diagnostic challenge. On the other hand, the study of the relapse risk markers after laparoscopic myomectomy is of high interest. We investigated the molecular phenotype of different types of leiomyoma after hysterectomy or laparoscopic surgery in reproductive and menopausal women. Standard immunohistochemistry was used to detect proliferation markers Ki67 and cyclin D1, apoptotic markers Bcl2 and Cas3, and ER and PR. The results of our study indicated that ER expression is significantly higher in relapsed leiomyoma, compared to control group. In addition, relapsed leiomyomas are characterised with high proliferation and apoptotic index. With regard to STUMP despite histological homogeneity, this entity is characterised with the presence of three distinct molecular subtypes, based on proliferation and apoptotic marker expression, which should be used as diagnostic aid in these tumors.

Keywords: relapse risk markers, laparoscopic myomectomy, smooth muscle tumors of uncertain malignancy potential, STUMP.

РЕЗЮМЕ

РЕЦИДИВИРУЮЩИЕ ЛЕЙОМИОМЫ И ГЛАДКОМЫШЕЧНЫЕ ОПУХОЛИ С НЕОПРЕДЕЛЁННЫМ ЗЛОКАЧЕСТВЕННЫМ ПОТЕНЦИАЛОМ – ФЕНОТИПИЧЕСКИЕ ОСОБЕННОСТИ У ЖЕНЩИН РЕПРОДУКТИВНОГО ВОЗРАСТА

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Лейомиомы матки - частые опухоли тазовой полости у женщин, из различных подтипов которых особенную диагностическую проблему представляют гладкомышечные опухоли с неопределённым злокачественным потенциалом. На сегодняшний день весьма актуально определить риск развития рецидива после лапароскопических миомэктомий. Изучены фенотипические особенности различных типов лейомиом у женщин репродуктивного и менопаузального возраста, которые получены путём гистерэктомии или лапароскопических миомэктомий. Стандартным иммуногистохимическим методом изучены молекулярные маркеры: пролиферативные маркеры Ki67 и cyclin D1, маркеры апоптоза Bcl2 и Cas3, ER и PR. Результаты исследования показали, что экспрессия ER значительно выше в рецидивирующих лейомиомах в сравнении с контрольной группой, а экспрессия PR - ниже. Рецидивирующие лейомиомы характеризуются высокой пролиферативной и низкой апоптозной активностью. Что касается гладкомышечных опухолей с неопределённым злокачественным потенциалом, несмотря на гистологическую однородность, в этой нозологии возможно выделение трёх молекулярных подтипов по характеру экспрессии пролиферативных и апоптозных маркеров, что необходимо учитывать при диагностике этого заболевания.

რეზიუმე

მორეციდივე ლეიომიომები და გლუვკუნთოვანი სიმსივნეები გაურკვეველი ავთვისებიანობის პოტენციალით - ფენოტიპური თავისებურებები რეპროდუქციული ასაკის ქალებში

თ.კიკნაძე, გ.თევდორაშვილი, თ.მუზაშვილი, მ.გაჩეჩილაძე, გ.ბურკაძე

თბილისის სახელმწიფო სამედიცინო უნივერსიტეტი, საქართველო

საშვილონოს ლეიომიომები წარმოადგენს მენჯის ღრუში განვითარებულ ხშირ სიმსივნეებს ქალებში, რომელთა მრავალი ჰისტოლოგიური ქვეტიპიდან, განსაკუთრებულ დიაგნოსტიკურ პრობლემას წარმოადგენს გლუვკუნთოვანი სიმსივნეები ავთვისებიანობის გაურკვეველი პოტენციალით. სადღეისოდ აქტუალურია ლეიომიომების რეციდივის განვითარების რისკის განსაზღვრა ლაპაროსკოპიული მიომექტომიის შემდეგ.

შესწავლილია ჰისტერექტომიით და ლაპაროსკოპიული მიომექტომიით მიღებული სხვადასხვა ტიპის ლეიომიომების ფენოტიპური მახასიათებლები რეპროდუქციული და მენოპაუზური ასაკის ქალებში. სტანდარტული იმუნოჰისტოქიმიური მეთოდით გამოკვლეულია ისეთი მარკერების ექსპრესია, როგორებიცაა პროლიფერაციული მარკერები Ki67 და cyclin D1, აპოპტოზური მარკერები Bcl2 და Cas3 და ER, PR. კვლევის შედეგებმა აჩვენა, რომ მორეციდივე ლეიომიომებში გაცილებით უფრო მაღალია ER-ის ექსპრესია შედარებით საკონტროლო ჯგუფთან, მაშინ როდესაც PR-ის ექსპრესია, პირიქით, დაბალია. მორეციდივე ლეიომიომები ხასიათდებიან მაღალი პროლიფერაციული და დაბალი აპოპტოზური აქტივობით. რაც შეეხება გლუვკუნთოვან სიმსივნეებს გაურკვეველი ავთვისებიანობის პოტენციალით, მიუხედავად ჰისტოლოგიური ერთგვაროვნებისა, ამ ნოზოლოგიაში შესაძლებელია სამი სახის მოლეკულური ქვეჯგუფის გამოყოფა პროლიფერაციული და აპოპტოზური მარკერების ექსპრესიის მიხედვით, რაც აუცილებელია გათვალისწინებული იყოს ამ დაზიანების დიაგნოსტიკაში.

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STEM CELL INDEX IN THE PROGRESSION OF CERVICAL INTRAEPITHELIAL NEOPLASIA

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Cervical cancer represents the fourth most common type of gynaecological malignancy worldwide. Cervical cancer is characterised with high mortality rate, especially in developing countries, such as Georgia [1]. The high risk human papillomaviruses (HR-HPV), represent the major etiologic factor for cervical cancer [2]. However, it has been noted that stem cells are also playing one of the major roles in the development and progression of cervical cancer [3]. There are many different markers of stem cells including CD44 [4]. CD44 represents the primary adhesion molecule, which is involved in many different biological processes [5]. Some, studies indicate that, CD44 expression is relatively higher in cervical cancer tissues, compared to normal, non-tumorous tissues [5]. In vitro studies indicate that, cervical cancer cells, which are positive for CD44, show higher proliferation and self-renewal properties [6]. CK17 is also considered as another potential marker for cervical cancer stem cells. In vitro studies show that after TGF-β stimulation of CK17 significantly increases the stem cell like properties in cervical cancer cells [7]. Despite the ample of in vitro data, there is the lack of studies, investigating stem cell markers in patient cervical cancer tissues. In addition, little is known with regards to the association of the presence of CD44/CK17 positive stem cell population with the progression of cervical intraepithelial neoplasia (CIN) into cancer, as well as their association with epithelial-mesenchymal markers and proliferation and apoptotic characteristics in CIN and cancer specimens. In our previous studies, we have shown that epithelial-mesenchymal transition, as well as a certain proliferation and apoptotic characteristics are significantly associated with the progression of CIN. In our current study, we decided to extend previous studies and investigate the role of stem cells in the progression of CIN. In addition, we have investigated the correlation between stem cell markers, such as CD44 and CK17 with epithelial-mesenchymal transition and proliferation and apoptotic characteristics in CIN and cervical carcinoma specimens.

Material and methods. Formalin fixed and paraffin embedded tissue material was retrieved from the Research, Diagnostic and Teaching Laboratory of Tbilisi State Medical University, Georgia. Study included altogether 140 tissue samples, divided into two major groups: cases without co-infections (n=54) and cases with co-infections (n=86). Co-infections included bacterial vaginosis, chlamydia trachomatis and candida albicans. Cases without co-infections were divided into following subgroups: normal cervix (10 cases), CINI (18 cases), CINII (14 cases), CINIII (7 cases), invasive carcinoma (5 cases); Cases with co-infections were divided into following subgroups: cervix with only infections (15 cases), CINI (29 cases), CINII (19 cases), CINIII (15 cases), invasive carcinoma (8 cases).

Immunohistochemistry. 4μ FFPE tissue sections were deparaffinized in xylene, rehydrated by using serial dilutions of ethanol (96%, 80%, 70%) and heat mediated antigen retrieval has been performed. Ready to use antibodies against the following antigens were used: Ki67 (K2), Cyclin D1 (polyclonal) and phosphohistone-H3 (pHH3), E-cadherin(36B5), p63 (7JUL), β-catenin (17C2), vimentin (V9) Cas3 (cleaved), BAX (E63) and ER (6f11) (Leica). Staining and visualisation has been performed using Bond polymer refine detection system. The expression of all markers was evaluated as the percentage of marker positive cells.

mRNA analysis from The Cancer Genome Atlas (TCGA). The raw gene expression (mRNA) data for ovarian cancer was downloaded from the www.firebrowse.org, the cohort included altogether 309 patients with different grades of ovarian carcinoma. The study relevant genes, including CD44, CK17, Ki67, ER, CAS3, BAX, P63, E-Cadherin, β-catenin have been identified with the search function. Cyclin D1 was excluded from the study due to the lack of significant correlations with any other marker on immunohistochemistry level. In addition to standard statistical analysis (below), the two step clustering of the data have been performed.

Comparisons between groups were made using Mann-Whitney U test and Kruskal-Wallis test. The Kruskal-Wallis test is a nonparametric (distribution free) test, and is used when the assumptions of one-way ANOVA are not met. The Kruskal-Wallis test can be used for both continuous and ordinal-level dependent variables. Correlations were assessed using Spearman's rank correlation. The Spearman's rank correlation is also used when data is non-parametrically distributed. P values <0.05 were considered as significant. All statistical tests were performed using SPSS software V20.00.

Results and discussion. The results of the study in specimens without co-infections showed the following distribution of CD44 and CK17 in study groups: the mean positivity of CD44 in normal cervix was 7±2.1, in CINI it was 15±5.3, in CINII it was 28±6.1, in CINIII it was 41±9.3 and in invasive carcinoma the mean positivity for CD44 was 57±8.6. With regards to CK17, in normal cervix the mean positivity was 68±9.1, in CINI the mean positivity was 32±3.6, in CINII the mean positivity was 43±6.8, in CINIII the mean positivity was 47±7.7 and in invasive carcinoma, the mean positivity for CK17 was 36±4.7.

The results of the study in specimens with co-infections showed the following distribution of CD44 and CK17 in study groups: the mean positivity of CD44 in normal cervix was 9±3.3, in CINI it was 16±6.2, in CINII it was 31±4.8, in CINIII it was 48±9.9 and in invasive carcinoma the mean positivity for CD44 was 63±13.3. With regards to CK17, in normal cervix the mean positivity was 71±7.1, in CINI the mean positivity was 36±4.8, in CINII the mean positivity was 47±9.7, in CINIII the mean positivity was 52±6.7 and in invasive carcinoma, the mean positivity for CK17 was 32±6.1.

The correlation analysis in specimens without co-infections, between CD44 and other markers showed the following results: There was significant positive correlation between CD44 and vimentin ($r=.894$, $p<0.001$), Ki67 ($r=.867$, $p<0.0001$), phosphohistone-H3 ($r=.821$, $p<0.0001$) and Bax1 ($r=.867$, $p<0.0001$), whilst there was a negative correlation between CD44 and Cas3 ($r=-.942$, $p<0.0001$), E-cadherin ($r=-.851$, $p<0.0001$) and β-catenin ($r=-.923$, $p<0.0001$). The correlation analysis between CK17 and other markers did not show any significant correlation on immunohistochemistry level.

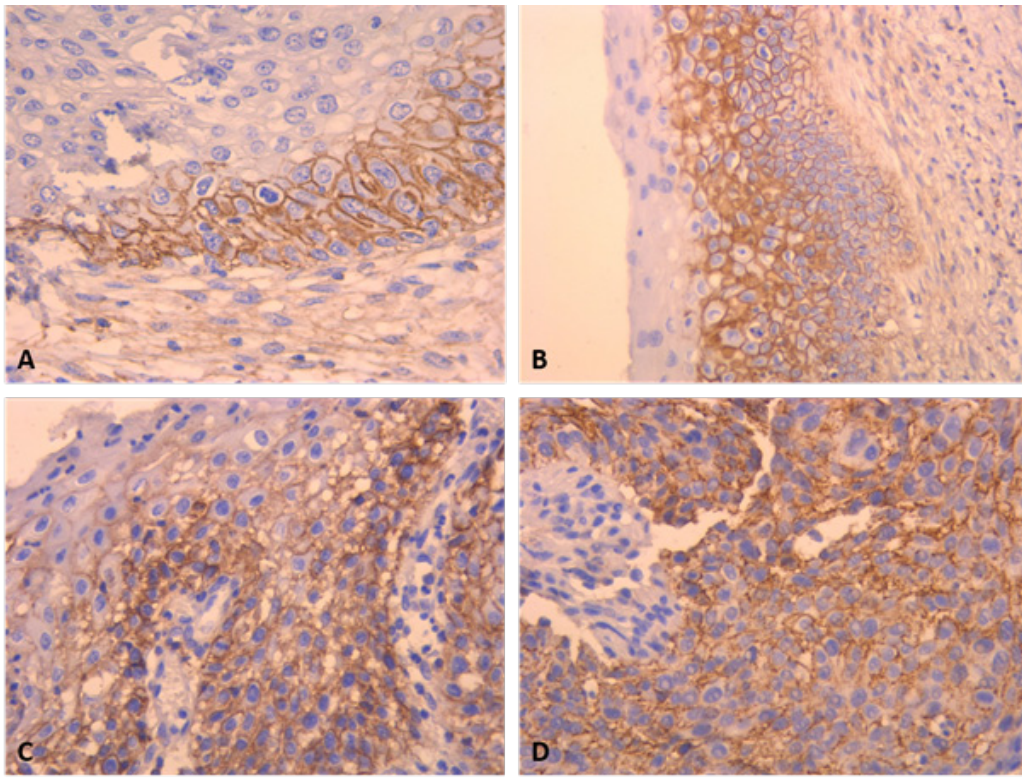


Fig. 1. The expression of CD44 in A. CINI, B. CINII, C. CINIII, D. Cervical Carcinoma, IHC, x200

Table 1. Distribution of CD44 and CK17 in specimens without co-infections

	Without co-infection		
	CD44 (%)	**CK17 (%)**	**Total N**
Normal cervix	7	68	10
CINI	15	32	18
CINII	28	43	14
CINIII	41	47	7
Invasive CA	57	36	5

Table 2. Distribution of CD44 and CK17 in specimens with co-infections

	With co-infection		
	CD44 (%)	**CK17 (%)**	**Total N**
Cervix with Infection	9	71	15
CINI	16	36	29
CINII	31	47	19
CINIII	48	52	15
Invasive CA	63	32	8

The correlation analysis in specimens with co-infections, between CD44 and other markers showed the following results: There was significant positive correlation between CD44 and vimentin (r=.914, p=0.041), Ki67 (r=.897, p<0.0001), phospho-histone-H3 (r=.873, p<0.0001) and Bax1 (r=.930, p<0.0001), whilst there was a negative correlation between CD44 and Cas3 (r=-.972, p<0.0001), E-cadherin (r=-.871, p<0.0001) and β-catenin (r=-.943, p<0.0001).

Table 3. Results of correlation analysis in specimens without co-infections

Spearman's rho		CK17	p63	E-cadherin	β-catenin	vimentin	Cas3	Bax1	Ki67	Cyclin D1	Phosphohiston-H3	ER
CD44	Correlation Coefficient	-0,300	0,000	-0,851	-0,923	0.894*	-0,942	0.890**	0.867**	0,000	0.8210**	-0,600
	Sig. (2-tailed)	0,624	1,000	0,000	0,000	0,041	0,000	0,000	0,000	1,000	0,000	0,285
	N	54	54	54	54	54	54	54	54	54	54	54
CK17	Correlation Coefficient		,200	,300	,300	-,112	,300	-,300	-,300	-,200	-,300	-,100
	Sig. (2-tailed)		,747	,624	,624	,858	,624	,624	,624	,747	,624	,873
	N		54	54	54	54	54	54	54	54	54	54
p63	Correlation Coefficient			0,000	0,000	-,224	0,000	0,000	0,000	-1.000**	0,000	,400
	Sig. (2-tailed)			1,000	1,000	,718	1,000	1,000	1,000		1,000	,505
	N			54	54	54	54	54	54	54	54	54
E-cadherin	Correlation Coefficient				0.956**	-.894*	0.820**	-0,861	-0,971	0,000	-1.000**	,600
	Sig. (2-tailed)				0,000	,041	0,000	0,000	0,000	1,000		0,285
	N				54	54	54	54	54	54	54	54
β-catenin	Correlation Coefficient					-.894*	0.870**	-0,93	-0,89	0,000	-0,84	,600
	Sig. (2-tailed)					,041	0,000	0,000	0,000	1,000	0,000	0,285
	N					54	54	54	54	54	54	54
vimentin	Correlation Coefficient						-.894*	.894*	.894*	,224	.894*	-.894*
	Sig. (2-tailed)						,041	,041	,041	,718	,041	,041
	N						54	54	54	54	54	54
Cas3	Correlation Coefficient							-0,91	-0,932	0,000	-0,862	,600
	Sig. (2-tailed)							0,000	0,000	1,000	0,000	0,285
	N							54	54	54	54	54
Bax1	Correlation Coefficient								0.970**	0,000	0.990**	-,600
	Sig. (2-tailed)								0,000	1,000	0,000	,285
	N								54	54	54	54
Ki67	Correlation Coefficient									0,000	0.844**	-,600
	Sig. (2-tailed)									1,000	0,000	,285
	N									54	54	54
Cyclin D1	Correlation Coefficient										0,000	-,400
	Sig. (2-tailed)										1,000	,505
	N										54	54
Phosphohiston-H3	Correlation Coefficient											-,600
	Sig. (2-tailed)											,285
	N											54

Table 4. Results of correlation analysis in specimens with co-infections

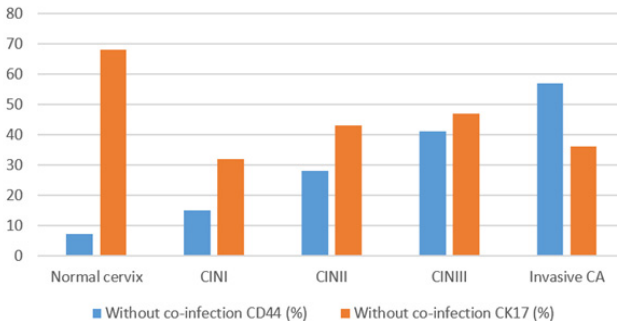
Spearman's rho		**CK17**	**p63**	**E-cad-herin**	**β-cate-nin**	**vi-men-tin**	**Cas3**	**Bax1**	**Ki67**	**Cyclin D1**	**Phos-pho-his-ton-H3**	**ER**
CD44	Correlation Coefficient	-0,400	0,000	-0,871	-0,943	0.914*	-0,972	0.930**	0.897**	0,000	0.873**	-0,700
	Sig. (2-tailed)	0,523	1,000	0,000	0,000	0,041	0,000	0,000	0,000	1,000	0,000	0,369
	N	86	86	86	86	86	86	86	86	86	86	86
CK17	Correlation Coefficient		,100	,200	,200	-,222	,500	-,200	-,200	-,200	-,600	-,900
	Sig. (2-tailed)		,642	,716	,716	,716	,716	,716	,716	,642	,716	,624
	N		86	86	86	86	86	86	86	86	86	86
p63	Correlation Coefficient			0,000	0,000	-,339	0,000	0,000	0,000	-1.000**	0,000	,700
	Sig. (2-tailed)			1,000	1,000	,621	1,000	1,000	1,000		1,000	,809
	N			86	86	86	86	86	86	86	86	86
E-cad-herin	Correlation Coefficient				0.978**	-0,924	0.864**	-0,893	-0,991	0,000	-1.000**	,300
	Sig. (2-tailed)				0,000	,041	0,000	0,000	0,000	1,000		0,385
	N				86	86	86	86	86	86	86	86
β-cate-nin	Correlation Coefficient					-0,934	0.880**	-0,96	-0,91	0,000	-0,85	,800
	Sig. (2-tailed)					,041	0,000	0,000	0,000	1,000	0,000	0,485
	N					86	86	86	86	86	86	86
vime-ntin	Correlation Coefficient						-0,994	-0,994	-0,994	,224	-0,994	-0,994
	Sig. (2-tailed)						,031	,031	,031	,718	,031	,031
	N						86	86	86	86	86	86
Cas3	Correlation Coefficient							-0,94	-0,952	0,000	-0,882	,500
	Sig. (2-tailed)							0,000	0,000	1,000	0,000	0,285
	N							86	86	86	86	86
Bax1	Correlation Coefficient								0.990**	0,000	0.990**	-,800
	Sig. (2-tailed)								0,000	1,000	0,000	,225
	N								86	86	86	86
Ki67	Correlation Coefficient									0,000	0.944**	-,700
	Sig. (2-tailed)									1,000	0,000	,215
	N									86	86	86
Cyclin D1	Correlation Coefficient										0,000	-,300
	Sig. (2-tailed)										1,000	,805
	N										86	86
Phos-pho-histon-H3	Correlation Coefficient											-,900
	Sig. (2-tailed)											,315
	N											86

Table 5. Results of correlation analysis from the TCGA dataset

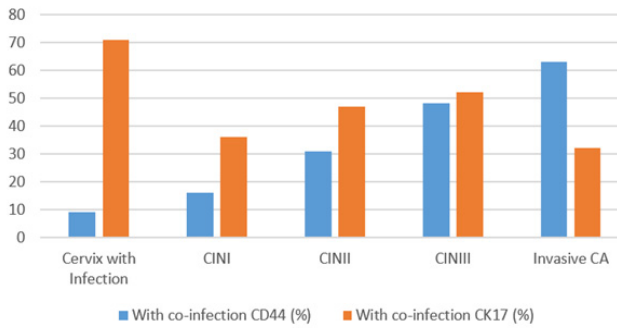
Spearman's rho		CK17	Ki67	ER	CAS3	BAX	P63	E-Cadherin	β-catenin	Vimentin
CD44	Correlation Coefficient	.370**	.112*	-.184**	-,019	-.172**	.569**	,087	-.115*	,008
	Sig. (2-tailed)	,000	,049	,001	,739	,002	,000	,129	,044	,882
	N	309	309	309	309	309	309	309	309	309
CK17	Correlation Coefficient		-.183**	-.270**	-,059	,023	.371**	,022	-,076	-,088
	Sig. (2-tailed)		,001	,000	,300	,686	,000	,702	,180	,124
	N		309	309	309	309	309	309	309	309
Ki67	Correlation Coefficient			,103	,077	-.383**	.152**	.141*	.277**	.136*
	Sig. (2-tailed)			,069	,175	,000	,008	,013	,000	,017
	N			309	309	309	309	309	309	309
ER	Correlation Coefficient				,033	-.162**	-.178**	-,065	,034	.196**
	Sig. (2-tailed)				,559	,004	,002	,253	,555	,001
	N				309	309	309	309	309	309
CAS3	Correlation Coefficient					.147**	.181**	-,007	-,089	-,002
	Sig. (2-tailed)					,010	,001	,904	,118	,972
	N					309	309	309	309	309
BAX	Correlation Coefficient						-.130*	-,085	-.220**	,112
	Sig. (2-tailed)						,022	,134	,000	,050
	N						309	309	309	309
P63	Correlation Coefficient							.120*	-.143*	-.156**
	Sig. (2-tailed)							,035	,012	,006
	N							309	309	309
E-Cadherin	Correlation Coefficient								,098	-,106
	Sig. (2-tailed)								,086	,063
	N								309	309
β-catenin	Correlation Coefficient									.175**
	Sig. (2-tailed)									,002
	N									309

Table 6. Distribution of different markers in CD44 low and high groups. Mann-Whitney U test. Data analysis results from TCGA

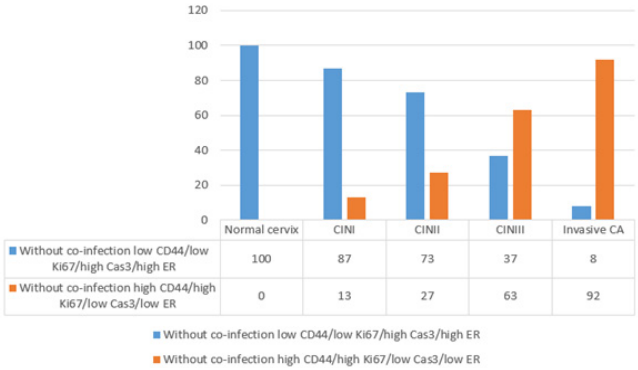
CD44	N	Mean Rank	*P*	Markers
Low	154	130.79	<0.0001	CK17
High	155	179.05		
Low	154	140.68	0.005	Ki67
High	155	169.23		
Low	154	170.23	0.003	ER
High	155	139.86		
Low	154	156.69	0.74	Cas3
High	155	153.32		
Low	154	172.19	0.001	BAX
High	155	137.92		
Low	154	110.93	< 0.0001	p63
High	155	198.79		
Low	154	144.81	0.046	E-cadherin
High	155	165.12		
Low	154	160.78	0.057	β-caenin
High	155	149.26		
Low	154	152.22	0.586	Vimentin
High	155	157.76		



Graph 1. Distribution of CD44 and CK17 in groups without co-infections



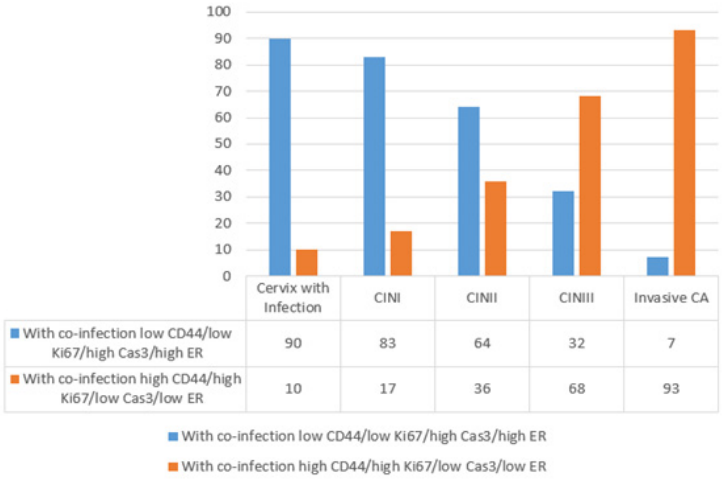
Graph 2. Distribution of CD44 and CK17 in groups with co-infections



Graph 3. The distribution of cervical lesions in two phenotypic groups, in specimens without co-infections

The mRNA expression data analysis showed the following significant correlations: CD44 was significantly, positively correlated with CK17 (r=.317, p<0.0001), Ki67 (r=.112, p=0.049) and p63 (r=.569, p<0.0001), whilst it was negatively correlated with ER (r=-.184, p<0.001), Bax (r=-.172, p=0.002) and β-catenin (r=-.115, p=0.044).

In addition to Spearman's correlation analysis, Mann-Whitney U test showed the following results in CD44 low and high groups: CD44 high group was characterised with the higher expression of CK17 (p<0.0001), higher expression of Ki67 (p=0.005), lower expression of ER (p=0.003), lower expression of BAX (p=0.001), higher expression of p63 (p<0.0001) and



Graph 4. The distribution of cervical lesions in two phenotypic groups, in specimens with co-infections

higher expression of E-cadherin (p=0.046). The comparative analysis of CD44 expression in groups without co-infections showed that the expression of CD44 is lower in normal cervix and it is significantly increased during the progression of cervical intraepithelial neoplasia, showing the maximum expression in invasive carcinoma. Whilst CK17 showed more heterogeneous distribution pattern. Particularly, the highest expression of CK17 was seen in normal cervical epithelium and the lowest expression of CK17 was seen in CINI. However, the expression of CK17 was also gradually increasing from CINI to invasive carcinoma of the cervix. The comparative analysis of CD44 and CK17 expression in groups with co-infections showed the similar trends. However, when compared the expression of CD44 and CK17 was more pronounced in groups with co-infections, compared to the groups without co-infections.

The further analysis of immunohistochemical expression of the mentioned markers identified two major groups of cervical intraepithelial neoplasia and invasive carcinoma, based on the expression of stem cell marker CD44 and proliferation, apoptotic and hormonal characteristics. First group was characterised with I. low CD44/low Ki67/high Cas3/high ER phenotype, whilst the second group was characterised with II. high CD44/high Ki67/low Cas3/low ER phenotype. Particularly, the phenotype I was seen in 100% of normal cervical tissues, without co-infections. Phenotype II, which indicates more aggressive characteristics of the lesion, was seen in 13% of CINI without co-infections, in 27% of CINII without co-infections, in 63% of CINIII without co-infections and in 92% of invasive carcinoma without co-infections.

Similar trend has been seen in specimens with co-infections. Particularly, the phenotype I was seen in 90% of normal cervical tissues, with co-infections. Phenotype II, which indicates more aggressive characteristics of the lesion, was seen in 17% of CINI with co-infections, in 36% of CINII without co-infections, in 68% of CINIII with co-infections and in 93% of invasive carcinoma with co-infections.

In addition to immunohistochemical data, we have performed the in silico gene expression analysis from TCGA dataset of 309 ovarian cancers, which showed the similar results. Particularly, the two groups were also clustered separately based on the expression of above mentioned markers. Phenotype I represented 7.5% of invasive cervical carcinomas, whilst phenotype I was characteristic of 93.5% of invasive cervical carcinomas. To the best of our knowledge we are first who performed such a profound analysis of stem cell marker CD44 and epithelial-mesenchymal, proliferation, apoptosis and hormone receptor markers in tissue specimens from the patients with cervical intraepithelial neoplasia and invasive cervical carcinoma. The results of our study indicate that the detection of CD44 stem cell marker might be used as an important prognostic factor for cervical intraepithelial neoplasia progression, together with proliferation marker Ki67, apoptotic marker Cas3 and ER. It is known that CINI lesions progressing into advanced CIN disease and cervical carcinoma in about 8-12% of cases, whilst the progression rate of CINIII into carcinoma is about 60%. Our study results are in line with this previous observation, as we have shown that CIN lesions with more aggressive phenotype (II) represent about 13-17% of CINI lesions and 63-68% of CIN III lesions. Therefore, the mentioned phenotypic characteristics could be used for the early assessment of CIN patient prognosis and for the relevant clinical management.

Conclusions. The results of our study indicate that stem cell index based on the CD44 detection is significantly increased with the progression of cervical intraepithelial neoplasia. In addition, CD44 expression significantly correlates with the epithelial-mesenchymal transition, proliferation-apoptotic features and ER status in both protein and mRNA level. Two, low and high risk groups of cervical intraepithelial lesions as well as carcinoma can be identified based on the expression of CD44, Ki67, Cas3 and ER.

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SUMMARY

STEM CELL INDEX IN THE PROGRESSION OF CERVICAL INTRAEPITHELIAL NEOPLASIA

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Stem cells represent the small subpopulation of healthy and cancerous tissues, which are characterised with increased proliferation and self-renewal properties. From the many different markers of stem cells, we have investigated the stem cell index during the progression of cervical intraepithelial neoplasia (CIN), based on the immunohistochemical expression of CD44 in total of 140 tissue samples from uterine cervix. In addition, we have performed the profound correlation analysis of CD44 with different epithelial-mesenchymal, proliferation, apoptosis and hormonal markers at both protein and mRNA level. The results of our study indicated that, stem cell index based on the CD44 detection is significantly increased with the progression of cervical intraepithelial neoplasia. In addition, CD44 expression significantly correlates with the epithelial-mesenchymal transition, proliferation-apoptotic features and ER status in both protein and mRNA level. Two, groups of cervical intraepithelial lesions as well as carcinoma can be identified based on the expression of CD44, Ki67, Cas3 and ER. To the best of our knowledge we are first to demonstrate such findings in CIN and cervical carcinoma and identified characteristics could be used for the early assessment of CIN patient prognosis and for the relevant clinical management.

Keywords: stem cell index, progression of cervical intraepithelial neoplasia, CIN, immunohistochemical expression of CD44.

РЕЗЮМЕ

ОСОБЕННОСТИ РАСПРЕДЕЛЕНИЯ СТВОЛОВЫХ КЛЕТОК ПРИ ПРОГРЕССИИ ИНТРАЭПИТЕЛИАЛЬНЫХ НЕОПЛАЗИЙ ШЕЙКИ МАТКИ

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Стволовые клетки являются малочисленной популяцией клеток как в нормальных, так и в опухолевых тканях, характеризуются высокой способностью к самообновлению и пролиферации. Среди многочисленных маркеров стволовых клеток, для изучения особенностей их распределения, авторами применена иммуногистохимическая экспрессия маркера CD44 на 140 тканевых образцах шейки матки. Проведен корреляционный анализ между CD44 и другими маркерами прогрессии интраэпителиальных неоплазий шейки матки, таких как эпителиально-мезенхимальные маркеры трансформации, пролиферативно-апоптозные маркеры и рецептор эстрогена.

Результаты исследования показали, что стволовоклеточный индекс значительно увеличивается в процессе прогрессии интраэпителиальных неоплазий шейки матки и достоверно коррелирует с маркерами эпителиально-мезенхимальной трансформации и пролиферативно-апоптозными, также как с ER статусом. В интраэпителиальных неоплазиях шейки матки возможно выделение двух различных фенотипных групп по экспрессии маркеров CD44, Ki67, Cas3 и ER, которые коррелируют с прогрессией интраэпителиальных неоплазий шейки матки. Данные проведенного исследования в дальнейшем возможно использовать для оценки риска прогрессии интраэпителиальных неоплазий шейки матки и разработки адекватного клинического менеджмента.

რეზიუმე

ღეროვანი უჯრედების განაწილების თავისებურებები საშვილოსნოს ყელის ინტრაეპითელური ნეოპლაზიის პროგრესიის დროს

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ღეროვანი უჯრედები წარმოადგენენ მცირე უჯრედულ პოპულაციას როგორც ნორმალურ,ისე სიმსივნურ ქსოვილებში, ხასიათდებიან თვითგანახლების და პროლიფერაციის მაღალი უნარით. ღეროვანი უჯრედების მრავალ მარკერებს შორის შესწავლილია ღეროვანი უჯრედების განაწილების თავისებურებები CD44-ის იმუნოჰისტოქიმიური ექსპრესიის მიხედვით 140 საშვილონოს ყელიდან აღებულ ქსოვილოვან მასალაში. ჩატარდა კორელაციური ანალიზი CD44-სა და საშვილონოს ყელის ინტრაეპითელური ნეოპლაზიის პროგრესიის სხვა მარკერებს შორის,როგორებიცაა ეპითელურ-მეზენქიმური ტრანსფორმაციის მარკერები, პროლიფერაციულ-აპოპტოზური მარკერები და ესტროგენის რეცეპტორი.

კვლევის შედეგებმა აჩვენა, რომ ღეროვანი უჯრედების ინდექსი მნიშვნელოვნად იზრდება საშვილოსნოს ყელის ინტრაეპითელური ნეოპლაზიების პროგრესიის პროცესში. ღეროვანი უჯრედების ინდექსი სარწმუნო კორელაციაშია ეპითელურ-მეზენქიმური ტრანსფორმაციის და პროლიფერაციულ-აპოპტოზურ მარკერებთან, ისევე, როგორც ER სტატუსთან. საშვილონოს ყელის ინტრაეპითელურ ნეოპლაზიებში შესაძლებელია ორი ფენოტიპურად განსხვავებული ჯგუფის გამოყოფა CD44, Ki67, Cas3 და ER ექსპრესიის მიხედვით, რომლებიც კორელაციაშია საშვილოსნოს ყელის ინტრაეპითელური ნეოპლაზიების პროგრესიასთან. კვლევის შედეგები შესაძლებელია მომავალში გამოყენებული იყოს საშვილონოს ყელის ინტრაეპითელური ნეოპლაზიების პროგრესიის რისკის განსაზღვრის და შესაბამისად ადექვატური კლინიკური მენეჯმენტის შემუშავების მიზნით.

PERIPHERAL NERVE LESIONS AFTER A MECHANICALLY INDUCED LIMB ISCHEMIA

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Non-compression damage to peripheral nerves of a limb is not a frequent trauma to extremities. Non-compression ischemia relates to lesions of a limb's great vessels (atherosclerosis, emboli, vasculitis, others). Frequently, they are consequences of a traumatic injury to an extremity [16]. A range of publications [17] contains selective descriptions of clinical cases of ischemic neuropathy, but the pathogenesis of damage to nerves of a limb remains understudied and unstructured. The reasons thereof are that the authors in their clinical researches describe separate cases with different etiology, while experimental studies made us able to investigate the development of damage to a limb's nerves and their recovery. Their main disadvantage was the focus on just a single factor as a task – ischemia of a nerve, while clinical cases are complicated with the probable presence of two – ischemia itself and pressure on the nerve. Moreover, the experimental researches mostly limit themselves by one or two models of isolated nerve compression [8, 10]. Although, such cases rarely occur in clinical practice, and the majority of the compression lesions are the consequences of a simultaneous trauma and compression to a limb with significant damage to skeletal muscles followed by their atrophy, tissue necrosis.

Acute necrosis of skeletal muscles and consequences thereof (myoglobinuria, impaired metabolism of electrolytes, acute renal impairment) are covered by scholars better, compared to the same of nerves of limbs. The conditions of peripheral nerves in compression and non-compression ischemia could reflect all the tissues of the extremity and the efficiency of the therapy in general. Besides, the literature sources lack enough data on the extent of the damage to muscles and peripheral nerves of an extremity in compression injury, and, which is the most important, their sensitivity to damage and stimulation of repairing processes.

In traumatology, the autologous tissue technologies are widely used, in particular for managing arthritis, traumas to a limb's nerves and tendons. Quite simple to obtain and use, and very promising are concentrates of platelet-rich plasma (PRP) and cells collected from the bone marrow aspirates (CBMA), and homogenized adipose tissue (HAT) derivable, especially in cases of peripheral neuropathies [10]. We found a range of researches with positive and promising results of the use thereof in the management of damages to the peripheral nervous system. The technology seems useful for the therapy of compression-ischemia neuropathies of the nerves in cases after a compartment syndrome.

Using experimental models of compression ischemia of a limb or peripheral nerves, we obtained a significant amount of data, but we should not equate all the results with the same of a human compression ischemia, so these disorders require further study.

The task of the research is to study damages to a sciatic nerve in mechanically-induced limb ischemia and after PRP, CMBA, and HAT injections.

Material and methods. *The background of the research is the animal model, a mechanically induced ischemia (MII)***.** The study involved Chinchilla rabbits (weighing 4.2-4.5 kg). The rabbits lived in standard cages with free access to food and water. We subdivided the animals into five groups, five animals each: group 1 (control) – intact animals; group 2 (MII) – animals with an injured limb; group 3 (MII+PRP) – animals with an injured limb treated by PRP; group 4 (MII+CBMA) – animals with an injured limb treated by CBMA; group 4 (MII+HAT) – animals with an injured limb treated by HAT.

MII was simulated by an elastic medical tourniquet (5.5 cm wide, eight units per an extremity), lasting through the middle third of a left hind limb, from the thigh to the ankle joint. It thereby immobilized the limb and caused impaired vascular perfusion of the skeletal muscles of the tibia (SMT). We removed the tourniquets after 6 hours.

The subfascial pressure. For the subfascial pressure measuring, we applied a classical invasive method *Whitesides* in an anterolateral sleeve of a shin, using a standard device *Stryker Intra-Compartmental Pressure Monitor* (USA) for a one-shot measurement of every value [2].

The cells suspensions preparation. The cell suspensions were injected into the upper third of the SMT once, 6 hours after the elastic tourniquet removal. Test animals got withdrawn for the experiment in 5th, 15th, and 30th days after it started with the limb MII (5 animals for each stage thereof).

The animals received anesthesia with 60 mg/kg sodium thiopental, intraperitoneal.

Platelet-rich plasma (PRP). We drew 5 ml of blood from the ear vein of a rabbit. By centrifuging the whole blood in an *Arthrex* tube (Arthrex ACP® Double Syringe System, Arthrex, Inc. USA) for 8 minutes at 760g, we obtained 1 ml of PRP under the layer of erythrocyte mass (the average platelet quantity was 928,000/ml).

Autologous concentrated bone marrow aspirate (CBMA). We aspirated 2 mL of bone marrow from the proximal part of an intact femur, with a 10G bone puncture needle and 5 mL syringe, like in the article [1]. To separate the aspirate, we used a Tulip Emulsifier filter (Tulip Medical Products, USA). After centrifuging of the aspirate with dextrose citrate coagulate – solution A (1:8) (Baxter C.A., USA/Belgium) for 8 minutes at 760g, we got 1.0 mL of supraerythrocytic fraction (the average cell quantity in native aspirate – 55.800/ml).

Homogenized adipose tissue (HAT). Through the access 2 cm, we took 5 mg of an abdominal gland and fined it as a suspension. For homogenization, the said suspension passed through a 1mm hole of two connected syringes.

Histological study. Sciatic nerves and SMTs of rabbits were fixed in 10% formalin solution in PBS (pH 7.4) for 24 hours at 4° C. The 15 μm slices of sciatic nerves, obtained with a cryotome, we impregnated with silver nitrate. On longitudinal sections of the nerve, we calculated the number of nerve fibers in the test area (the transverse axis of the nerve – 420 μm, in the micrograph – 420×550 μm; 0.23 mm^2).

Electron microscopy. The samples of tibial nerve were fixed in 2.5% solution of glutaraldehyde in phosphate buffer with 1% osmium tetrachloride, dehydrated in increasing concentrations of ethanol and acetone. The tissue samples we embedded in

the Epone-Araldite mixture. To get the ultrathin slices, we applied an ultratome (*Reihart*). For contrast, we used 2% sodium citrate and uranyl acetate. To analyze the sections obtained, we scanned them with an electron microscope *Tescan Mira 3 LMU* (CzechRepublic) (STEM detector).

Statistical analysis. The data obtained appeared as mean values (M) ± standard errors of the means (SEM) and have been compared using Mann-Whitney U-test. We took the differences as significant at $P < 0.05$.

Bioethics. All manipulations with the animals complied with the European Convention for the protection of vertebrate animals used for experimental and other scientific purposes, # 123, Council of Europe, L222, 24/08/1999, p. 31and the Commission on Bioethics of the State Institution "The Institute of Traumatology and Orthopedics under NAMS of Ukraine.".

Result and discussion. *Macroscopic studies.* After the MII, limb muscles were specific with acute edema and aggregation of intestinal tissue. After the incision of the fascia, we registered the emission of 0.5 to 2.0 ml of the liquid. Large and medium subfascial vessels on the knee level and shin muscles *(tibialis anterior, flexor digitorumlongus, gastrocremius)* were visually full with excessive blood, with the areas of hemorrhagic leakages in muscle sections. The texture of shin muscles changed: the surface muscles were edematous, soft, decolorized (from pink to yellowish-white). The deep muscles were more elastic and pink. On the 15th day, the extent of the edema was lower, compared to the 5th day, and the tissue of the muscles – more elastic, while in 30th days after the experiment started, there was no excessive interstitial fluid anymore. The color of the muscle tissues changed towards pinky-yellow, yellowish-white. The group of animals treated with PRP and CBMA had no injection areas; in the group of HAT, the areas of the insertion of the suspension were clear, specific with the excessive formation of connective tissues and angiogenesis, evidencing encapsulation of the aspirate. In all the animals with MII, we have detected the preservation of the integrity of sciatic nerves. The nerve was in the intramuscular space, without any signs of fibrosis processes along with it. The vessel network of the nerve and paraneural surroundings were distinct, the vessels full in blood, but without signs of hemorrhagic penetration into the intramuscular space. In other words, the mechanical damage to muscles of the shin, aggregation on interstitial fluid, and muscle tissue edema occur after the MII, decrease after the 5th day, and get replaced with necrotic changes in muscles without significant lesions to the sciatic nerve.

Microscopic changes in peripheral nerves of a limb. In longitudinal sections of nerves, we studied the structural changes of its fibers in the sciatic nerve fascicules. The nerve's integrity was intact, the same for the fascicular structure. The sheaths of nerves had no reasonable damages, except for epineurium. We have noticed an increased density of fibroblasts, evidencing cellular reactions in the stromal elements of the nerve, cell proliferation in the epineurium. Nerve fibers were present in the nerve's fascicules (Fig. 1), but their density had no significant differences within the period of the experiment (5, 15, and 30 days) and in terms of control. However, we have revealed deformed fibers with the different thickness of their myelin sheaths. On the 30th day after the MII, we identified "free" neurolemmocytes along the nerve fibers.

The distortion of a myelin sheath ultrastructure confirmed itself under electron microscopy (STEM). We studied the samples of the tibial nerve. On the 5th and 15th day, we noticed the impairment in the density of myelin lamellae, myelin sheath deformities, and necrotized fibers (Fig. 2). In the impaired myelin fibers, the axial cylinders could be intact (with microtubules, mitochondria, vesicles) or exfoliated from the myelin. The cytoplasm of neurolemmocytes contained atypical lamellar (membrane) structures, the primary pathologic feature of autophagy. Despite degeneration ovoids, appearing after the total disintegration of a myelin fiber, the cytosolic lamellar inclusions result from autophagy, alternation of the damaged myelin in neurolemmocytes, and are the signs of dystrophy [14]. The PRP, CBMA, and HAT groups had no significant difference in the extent of the damage to the nerve. All the samples studied confirmed the demyelination and necrosis. The changes in the myelin fibers were not specific and frequently diffused. Consequently, we concluded no dependence on the level of the damage in the separate fascicules.

We have not confirmed severe nerve fibers degeneration in part of sciatic nerve, although disclosed the trend to the reduction in their density (Fig. 3). In PRP, CBMA, and HAT groups, we registered no "free" neurolemmocytes. This fact can be the evidence of less damage to the nerve fibers after the MII; there were no clear morphologic signs of the nerve fibers' progressive degeneration in terms from 5th day to 30th day. It only means that the demyelination and degeneration of isolated nerve fibers occurs on the MII level and distally away from the compressive ischemia level, in particular in tibial nerve.

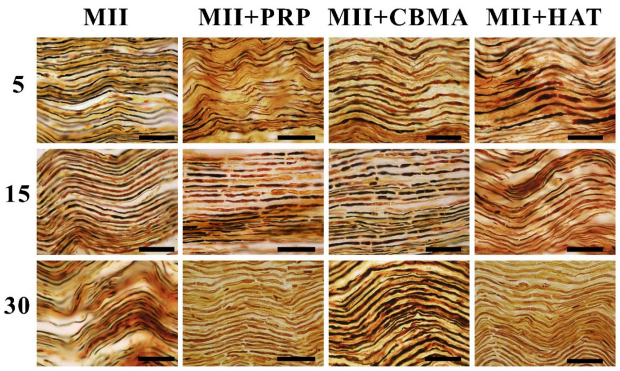

Fig. 1. A sciatic nerve of a rabbit on the 5th, 15th, and 30th day after the MII and the therapy. The impregnation with silver nitrate, scale bar 100 μm

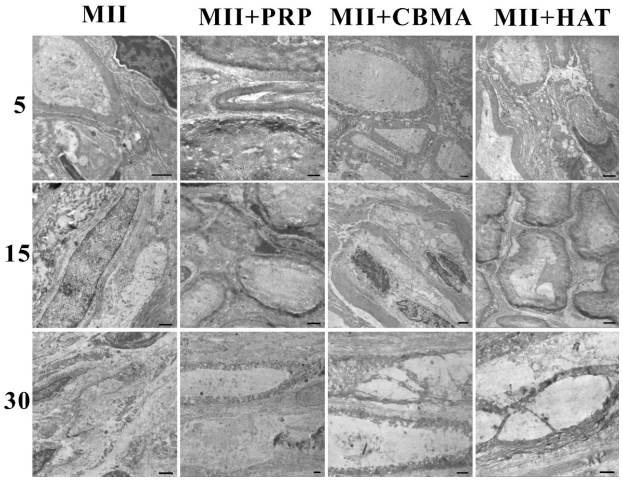

Fig. 2. A tibial nerve of a rabbit on the 5th, 15th, and 30th day after the MII and the therapy. Electron microscopy, scale bar 1 μm

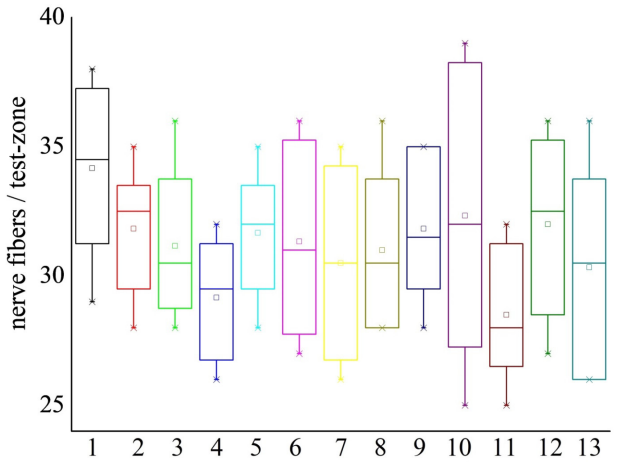

Fig. 3. The average amount of nerve fibers in the test-zones of a sciatic nerve after the MII and the injections of PRP, CBMA, and HAT

Note: 1 – control group; 2, 3, and 4 – MII on the 5th, 15th, and 30th day; 5, 6, and 7 – MII+PRP on the 5th, 15th, and 30th day; 8, 9, and 10 – MII+CBMA on the 5th, 15th and 30th day; 11, 12, and 13 – MII+HAT on the 5th, 15th, and 30th day

Analyzing the results of our own studies and the published articles, we concluded that muscles and peripheral nerves of a limb have different resistance against the compression ischemia. The skeletal muscles of a limb obviously endure significant damage from its compression. At the same time, the two factors, compression, and ischemia affect the limb's muscles and nerves, but it is not always possible to distinguish between the morphologic consequences of both ischemic injury and compression ischemia, as the expressions of the cellular necrosis are non-specific.

Descriptions of damage to muscles, their dystrophy, and slight signs of regeneration appear already in publications. In the focus of our interest, we have another aspect of compression of a limb, namely peripheral nerve conditions. We assumed that large nerves of extremities, like a sciatic one, could avoid suffering from the necrosis thanks to their specific anatomical position in the limb. Perhaps, their deep anatomical placement in an intramuscular space of a limb can prevent severe compressive damage. We also investigated how PRP, CBMA, and HAT therapy can influence these changes.

The analysis of clinical cases showed that the loss of nerve fibers in chronic non-compression ischemia is not critical; it may be less than 5% of myelin fibers. The basic expressions of the disorder are disproportionally thin myelin sheath, demyelination of the fibers, myelin fibers deformities [13]. The reduced speed of nerve impulses is thus predetermined by an axonal degeneration rather than by a demyelination process [6]. However, spontaneous recovery is also possible. Thus, Hitoshi Nukada et al. have described regenerating nerve fibers in the ischemically damaged nerves of a limb after 1-6 months [13]. Damage to nerves and further nerve fibers degeneration can occur without a significant muscle injury [18]. This fact could be explained by the higher vulnerability of nerve fibers to ischemia, compared to the muscle tissue [12]. Thus, in critical ischemia of a lower limb, Kudykin M.N. et al. reported muscle hypotrophy in 40% of cases, but neuropathy – in 100% [7].

In an experimental paper, a ligature to an aorta does not cause total muscle ischemia due to the collateral blood flow; muscles and nerves suffer equally upon such conditions [3]. According to other data, in non-compression ischemic neuropathies, degenerative muscle changes could progress without significant necrosis of muscles, confirming the hypothesis that human distal nerve fibers, compared to muscle tissues, are more vulnerable to the acute non-compression ischemia of limbs [18]. After the limited microemboli of limb arteries, was reported the necrosis of small groups of muscle fibers, but muscles regenerate further [4]. In such cases, regeneration launches one month after the injury, while muscle fibrosis does not develop in non-compression ischemia.

Having analyzed the literature, we assumed that the extent of the nerve lesion depends directly on the value of compression of a limb by a tourniquet applied to the extremity. We came to this conclusion on the background of the absence of acute necrosis to structural elements of the sciatic nerve after 6 hours of the limb's compression, while degeneration, destruction of nerve fibers' myelin expressed brightly on the level of the tibial nerve, topographically equal to and distally from the compression area. The data described vary in different authors; this relates to the various methods used for study, like only electrophysiological or morphological. Thus, if the compression ischemia lasts more than 2 hours, the extent of degeneration progresses [3], while in other cases described, 12-hours compressing ischemia leads only to endoneurial edema [9]. Compression with a tourniquet on the level of 250 mm Hg leads only to a reversible nerve blockade, at 500 mm Hg – rarely to demyelination, while at 1000 mm Hg – to demyelination and mechanical damage to the nerve [3]. In the research, we determined the subfascial pressure, 30-70 mm Hg at the 3rd-6th hour of the MII. The discovered difference in the damage to the sciatic and tibial nerves indicates the difference in subfascial and intra-tissue pressures, reflected in more significant damage to a tibial nerve, due to its anatomic peculiarities placed more superficially, compared to the sciatic one.

During the first 4-6 hours after the tourniquet removal, the nerve conduction remained, while 8 hours later is did not restored [9]. Hence, on the background of these periods, one can say about a "therapeutic window" in compressive ischemia management. During the same time, we inserted PRP, CBMA, and HAT into the skeletal muscles of the shin. Histological and morphometrical studies showed neither positive nor negative results of the insertion of tissue suspension into the damaged muscles.

In all the samples of the nerves, we saw different types of damages: unequal thickness and myelin deformities, atypically thin fibers, degeneration of the axial cylinders, lamellar structures in neurolemmocytes, partial destruction of a nerve's endoneurium. These changes were not all out; we have observed just a tendency to the decreased density of nerve fibers in the sciatic nerve on the 30th day after the MII, although the dystrophy of nerve fibers has already been noticed distally, in the tibial nerve. In other research, Richards R.L. described a significant increase in endoneurial collagen, progressing till complete replacement of the nerve bundles with collagen [5]. However, we have not discovered fibrosis in our own studies (except for the destruction of endoneurium and perineurium, isolated cell-less areas rich in collagen). We assessed all revealed disorders as the consequences of damage to the limb's blood vessels, because the injuries to vessels (stasis, thrombosis, lytic cell death in vessels) occurred as in muscles' myons, as in the capillaries of intramuscular space (unpublished data). However, the changes did not reach the total degeneration of the nerve. This fact could have several explanations. The blood supply to a limb's peripheral nerve is multilevel, and the vessels cover reasonably the areas on different levels. Damage to a nerve due to a focal loss of perfusion has been considered before as highly improbable because collateral vessels ensure proper blood circulation in the nerve [5]. At the same time, the difference between vascularization and revascu-

larization of a limb muscles exist. Thus, "fast muscles" (*soleus*) have a higher density of micro-vessels, more of them per muscle fiber, are longer, and with better VEGF expression, compared to the "slow muscles" (*extensor digitorum longus*). The explanation is in diverse physiology of various types of fibers in these muscles, their need for different oxygenation of the "fast" and "slow" aerobic and "fast" anaerobic fibers [15]. In our experiment, we neglected this division, as the compression ischemia was significant, non-selective, and led to the total necrosis of peripheral myons in all the muscles of a shin.

Consequently, the compression ischemia of a limb causes mild or moderate damage to the peripheral nerves of a limb, depending on the level of compression. Inserting PRP, CBMA, and HAT into the ischemic limb muscles showed no reliable effect on the development of structural changes in peripheral nerves while studying these processes in the ischemically damaged muscle fibers seems promising.

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SUMMARY

PERIPHERAL NERVE LESIONS AFTER A MECHANICALLY INDUCED LIMB ISCHEMIA

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The article describes the results of studying the changes in peripheral nerves of a limb after a mechanically induced ischemia. It assessed myelinated nerve fibers in sciatic and tibial nerves after 6 hours of ischemia, simulated by a tourniquet on the level of a tibial, knee, and a lower third of a femur. Fasciculi of the sciatic nerve have shown no changes in the density of nerve fibers in 5th, 15th, and 30th days after the simulated ischemia, but we revealed the deformed fibers with the different thickness of myelin sheath. In a tibial nerve, myelin loss and deformations occurred on the 5th and 15th day, and atrophy of nerve fibers – on the 15th and 30th days. Neurolemmocytes of the injured myelin nerve fibers demonstrate the signs of dystrophic processes and autophagy. After the insertion of platelet-rich plasma, concentrated bone marrow aspirate, and homogenized adipose tissue in the ischemically damaged muscles, not a significant difference appeared between the extent of damage to the nerve. Consequently, the structural signs of the damage to peripheral nerves depend more on the level of injury to a limb than on therapy with autologous cellular technologies.

Keywords: ischemia of limb, peripheral nerve, morphometry, platelet-rich plasma, concentrated bone marrow aspirate, homogenized adipose tissue.

РЕЗЮМЕ

ПОВРЕЖДЕНИЯ ПЕРИФЕРИЧЕСКИХ НЕРВОВ КОНЕЧНОСТИ ПРИ МЕХАНИЧЕСКИ-ИНДУЦИРОВАННОЙ ИШЕМИИ

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В статье описаны результаты исследования изменений периферических нервов конечности после механически индуцированной ишемии. Оценены миелиновые нервные волокна в седалищном и большеберцовом нервах после 6-ча-

совой ишемии, моделированной жгутом на уровне голени, коленного сустава и нижней трети бедра. В фасцикулах седалищного нерва изменений плотности нервных волокон на 5, 15 и 30 сутки после моделирования ишемии не выявлено, однако обнаружены деформированные волокна с разной толщиной миелиновой оболочки. В большеберцовом нерве на 5 и 15 сутки установлены демиелинизация и деформация миелина, а на 15 и 30 сутки – атрофия нервных волокон. В нейролемоцитах поврежденных миелиновых нервных волокон выявлены признаки дистрофических процессов и аутофагии. После введения в ишемически поврежденные мышцы тромбоцитарной плазмы, концентрата клеток аспирата костного мозга и гомогенизированной жировой ткани существенной разницы в степени повреждения нерва не выявлено. Сделано заключение, что структурные признаки повреждения периферических нервов конечности после ишемии являются неспецифическими и в большинстве случаев зависят от уровня повреждения конечности, чем от терапевтических подходов с использованием аутологических клеточных технологий.

რეზიუმე

კიდურის პერიფერიული ნერვების დაზიანებანი მექანიკურად ინდუცირებული იშემიის დროს

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სტატიაში წარმოდგენილია კიდურის პერიფერიული ნერვების ცვლილებების კვლევის შედეგები მექანიკურად ინდუცირებული იშემიის შემდეგ. შეფასებულია მიელინიანი ნერვული ბოჭკოები საჯდომ და დიდი წვივის ნერვებში 6-საათიანი იშემიის შემდეგ, რაც მოდელირებული იყო ლახტის გადაჭერით წვივის,მუხლის სახსრის და ბარძაყის ქვედა მესამედის დონეზე. საჯდომი ნერვის ფასციკულებში ნერვული ბოჭკოების სიმჭიდროვის ცვლილებები იშემიის მოდელირებიდან მე-5,მე-15 და 30-ე დღეს არ გამოვლინდა,თუმცა,აღმოჩენილია დეფორმირებული ბოჭკოები მიელინის გარსის სხვადასხვა სისქით. დიდი წვივის ნერვში მე-5 და მე-15 დღეს დადგენილია დემიელინიზაცია და მიელინის დეფორმაცია, ხოლო მე-15 და 30-ე დღეს – ნერვული ბოჭკოების ატროფია. დაზიანებული მიელინიანი ნერვული ბოჭკოების ნეიროლემოციტებში გამოვლინდა დისტროფიული პროცესების და აუტოფაგიის ნიშნები. იშემიურად დაზიანებულ კუნთებში თრომბოციტული პლაზმის, ძვლის ტვინის ასპირატის უჯრედების კონცენტრატის და ჰომოგენიზებული ცხიმოვანი ქსოვილის შეყვანის შემდეგ არსებითი განსხვავება ნერვის დაზიანების ხარისხში არ აღინიშნა. ავტორები დაასკვნიან, რომ კიდურის პერიფერიული ნერვების დაზიანებების ნიშნები იშემიის შემდეგ არასპეციფიკურია და, უმეტეს შემთხვევაში, მეტადაა დამოკიდებული კიდურის დაზიანების ხარისხზე, ვიდრე თერაპიულ მიდგომებზე აუტოლოგიური უჯრედული ტექნოლოგიების გამოყენებით.

ENZYMATIC ACTIVITY IN MICROSOMES, LIPID PEROXIDATION OF MICE HEPATOCYTES UNDER THE SODIUM FLUORIDE

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Fluorine is one of the most widespread and necessary microelements for the body of animals and humans, which is required in a clearly limited amount [9,10]. The biological role of this microelement is difficult to overestimate, since it is part of the tooth enamel, bones, participates in the formation of dentin, and also prevents the development of osteoporosis with age. For many biological processes, it serves as a catalyst or inhibitor [7,11,15].

Different concentrations of fluorine can affect the state of lipid peroxidation, as well as the functional state of the microsomes of liver hepatocytes. With prolonged ingestion, fluoride can lead to fluorosis [6,10,13,14].

The question of the physiological role of fluorine on the body remains open, since an excess of this trace element in the composition of water is the cause of the destruction of tooth enamel, inhibition of phosphorus-calcium and carbohydrate metabolism, and the activity of a number of enzymes. Due to this, fluorine can lead to the suppression of some intracellular processes in the body, reduce the activity of the immune system [5]. Acute fluoride intoxication leads to a decrease in the intensity of tissue respiration, the formation of reactive oxygen species by cells increases, lipid peroxidation processes increase, and the activity of antioxidant defense enzymes decreases. With the alimentary pathway of sodium fluoride entering the body, its negative effect affects the structure and function of the digestive system, primarily the liver [1].

It was found that the effect of sodium fluoride leads to an increase in all parameters of microsomal oxidation of hepatocytes, with the exception of cytochrome b5 [2-5,8]. This circumstance allows us to put forward the idea of stimulating free radical processes leading to disruption in the structure of hepatocyte membranes, oxidative destruction of biologically active substances [4].

Material and methods. The studies were carried out on sexually mature Wistar rats weighing 180-220 g. The animals were subjected to oral priming using a probe with an aqueous solution of sodium fluoride, once a day for 60 days in doses of 1/10, 1/100 and 1/1000 DL50, which was 20 mg/kg, 2 mg/kg and 0.2 mg/kg body weight (median lethal dose of active substance was 200 mg/kg when administered orally). The rats of the control group received appropriate volumes of drinking water. Each group consisted of 10 animals, the studies of indicators were carried out on days 10, 20, 30, 50 and 60 after the start of the experiment. Euthanasia was performed by decapitation with a guillotine knife after preliminary anesthesia with sodium thiopental in an amount of 50 mg/kg.

Confirmation of the induction of free radical processes by sodium fluoride was carried out using a chemiluminescent reaction of blood serum in the spectrum range of 400-600 nm. The amount of diene conjugates in rat liver tissue homogenates was assessed spectrophotometrically at 233 nm with preliminary extraction of a heptane-isopropanol mixture. The calculations were performed based on the molar extinction coefficient $\varepsilon=2.2\times105$ mol^{-1} cm^{-1}.

The content of TBA reactants in rat liver tissue homogenates was determined by the reaction of malonic dialdehyde and thiobarbituric acid (TBA). The amount was calculated based on the extinction coefficient $\varepsilon=1.56\times105$ mol^{-1} cm^{-1}.

The level of cipher bases, which are products of the interaction of carbonyl compounds with amino acids, amino groups of proteins, and nucleic acids, was determined in homogenates of liver tissue using a spectrofluorometer at a wavelength of 430 nm with preliminary extraction with a Folch mixture (chloroform-methanol).

Subcellular liver fractions were isolated by differential centrifugation. To obtain microsomes, the supernatant was centrifuged for 1 hour at 18000 g, the obtained precipitate was washed and suspended in the isolation medium (the protein content in the microsome suspension was 15-20 mg/ml). The activity of NAD (P) H-cytochrome c reductase in the suspension of rat liver microsomes was estimated in the presence of an electron acceptor cytochrome c, using a spectrophotometer at 30ºC and a wavelength of 550 nm. The enzyme activity was calculated using the coefficient of molar extinction of cytochrome c, which was equal to $18,5\times10^3$ $cm^{-1}M^{-1}$. The content of cytochrome P-450 in the suspension of rat liver microsomes was determined spectrophotometrically.

Statistical analysis of the results was carried out using the Statistica10 program.

Results and discussion. Free radical processes in the rat liver was assessed by the intensity of H2O2-induced chemiluminescence (CL). The enzymatic state of rat liver microsomes was initially assessed by the activity of NAD (P) H-cytochrome c reductase in the dynamics of observation for 10, 20, 30, 50, 60 days with the introduction of sodium fluoride in doses of 1/10, 1/100 and 1/1000 DL50 (Table 1, 2).

On days 10 and 20 of the experiment (Table 1), a statistically significant ($p<0,001$) increase in the level of the indicator under the influence of sodium fluoride at a dose of 1/10 DL50 was found, respectively, by 37 and 134%, in relation to the control group; by 37% of CL intensity in relation to the value in the previous observation term, in comparison with the control group, an increase was observed by 66%.

Of interest is the fact that on the 60th day of oral administration of sodium fluoride at a dose of 1/10 DL50, a statistically significant ($p<0,001$) decrease by 33% in the level of CL intensity of the liver of rats is observed in comparison with the control group. At a dose of 1/100 DL50, a significant ($p\leq0,002$) increase in the intensity of ultra-weak luminescence was observed in all periods of observation and was especially pronounced on the 30th day, on average it was 85%. Further, the level of CL intensity in the rat liver gradually decreased and on the 60th day it was 19%.

The results of the activity of NAD (P) H-cytochrome c reductase in the microsomal fraction of the liver of rats when exposed to sodium fluoride at subtoxic doses showed that on days 10 and 20 of oral administration of sodium fluoride at a dose of 1/10 DL50, statistically significant ($p\leq0,002$) was observed in Compared with the control group, a gradual increase in the activity of NADPH-cytochrome c reductase by 24 and 36%, respectively (Table 2). On the 30th day, there was a tendency towards a decrease in the enzyme activity, but in relation to the control group, the activity remained significantly ($p<0,001$) increased by 29%. After this period, a statistically significant ($p<0,001$) decrease was observed in comparison with the control group in the activity of NADPH-cytochrome c reductase by 20 and 30%, respectively, on days 50 and 60 of the action of sodium fluoride at a dose of 1/10 DL50.

Oral administration of sodium fluoride at a dose of 1/100 DL50 caused a significant ($p\leq0,004$) increase in the microsomal fraction of hepatocytes in the activity of NADPH-cytochrome c reductase by 6.26 and 32%, respectively, on the 10, 20, 30th day of the experiment. On the 50th day, there was a significant decrease in the activity of NADPH-cytochrome c reductase (by an average of 40%) in relation to the indicator on the 30th day, but in comparison with the control group, there was a slight statistically significant ($p <0,001$) increase in the indicator by 19%. On day 60, a dose of 1/100 DL50 resulted in a 20% decrease in the activity of NADPH-cytochrome c reductase.

The effect of sodium fluoride at a dose of 1/1000 DL50 did not cause a statistically significant effect of NADPH-cytochrome c reductase in relation to the control group on days 10 and 20. However, on days 30, 50, 60, there was a slight significant ($p\leq0,005$) increase in the enzyme activity by an average of 4-6%.

In microsomes of rat hepatocytes, oral administration of sodium fluoride at a dose of 1/10 DL50 led to an increase ($p <0,001$) in comparison with the control group in the activity of NADH-cytochrome c reductase by 10.23.31 and 20%, respectively, by 10, 20, 30. 50 days. On day 60, there was a statistically significant ($p <0,001$) decrease in the activity of NADH-cytochrome c reductase by an average of 39%. The effect of sodium fluoride at a dose of 1/100 DL50 was accompanied by a gradual increase ($p\leq0,038$) in the activity of microsomal NADH-cytochrome c reductase by 5, 11, 2 and 30%, respectively, on days 10, 20, 30, 50 of observation. On day 60, the value of the indicator decreased in relation to the preliminary term of the experiment by an average of 38%, but in relation to the value of the control group increased by 18% ($p<0,001$). The action of sodium fluoride in 1/1000 DL 50 practically did not cause changes in the activity of microsomal NADH-cytochrome c reductase in rats in comparison with the control group. Only on the 30th and 50th days there was an insignificant 4-5% ($p=0,013$ and $p=0,045$) increase in enzyme activity.

When analyzing the results obtained, it can be argued about the violation of the reductase activity of microsomes of hepa-

tocytes of experimental animals receiving sodium fluoride at a dose of 1/10, 1/100 DL50 and, as a consequence, the functioning of electron transport systems. The revealed changes on the 60th and in some cases on the 50th day are associated with the rearrangement of the lipid environment of microsomal reductases due to the possible initiation of the formation of a significant amount of reactive oxygen species according to literature data [16]. An increase in the activity of NAD (P) H-cytochrome c reductase in rats upon administration of sodium fluoride for a month can be considered as a protective-compensatory reaction and as a reason for a faster flow of electrons from reduced forms of NAD (P) H to cytochromes P-450 and b5 and significant formation of reactive oxygen species.

The dynamics of changes in the intensity of CL in the liver of rats as a percentage of the control group is shown in figure 1. The dynamics of the activity of NADPH reductase in microsomes of rat hepatocytes during toxification with sodium fluoride at doses of 1/10 and 1/100 DL50 is shown in Fig. 2 and 3.

The intensity of lipid peroxidation in the liver of rats, which were injected for a long time with sodium fluoride in doses of 1/10 and 1/100 DL50, was judged by the amount of its molecular products - diene conjugates of TBA reactants and chiffon bases (Table 3).

Table 1. Intensity of H2O2-induced chemiluminescence in rat liver homogenates upon exposure to sodium fluoride at a subtoxic dose (n = 10; Me [25%; 75%] or M±s)

Day of observation	Chemiluminescence intensity, pulse / s		
	Control	dose, DL50	
		1/10	1/100
10	294±23,5	403±29,5 p<0,001	398 [320; 418] p=0,002
20	296 [280; 335]	707±26,2 p<0,001	428 [415; 440] p<0,001
30	321±14,0	650 [638; 689] p=0,003	598 [575; 607] p<0,001
50	317±18,1	539 [498; 550] p<0,001	445 [432; 487] p<0,001
60	343±14,5	229±19,9 p<0,001	409±19,7 p<0,001

note: p is the level of statistical significance in relation to control

Table 2. Activity of NAD (P) H-cytochrome c reductase in the microsomal fraction of rat liver under the influence of sodium fluoride at subtoxic doses (n = 10; Me [25%; 75%] or M ± s)

Dose	Day of observation	NADPH-cytochrome c reductase, nM cytochrome c/mg protein • min	NADH-cytochrome c reductase, nM cytochrome c/mg protein • min
1/10 DL50	10	234±3,82 p<0,001	1027 [1017; 1030] p<0,001
	20	261±4,27 p=0,002	1156±9,45 p<0,001
	30	241 [240; 245] p<0,001	1233 [1228; 1245] p<0,001
	50	149±4,74 p<0,001	1115±11,0 p<0,001
	60	127±4,89 p<0,001	590±43,1 p<0,001
1/100 DL50	10	198 [195; 210] p=0,004	988 [945; 1005] p=0,038
	20	241 [238; 244] p<0,001	1036 [1028; 1056] p<0,001
	30	249±4,83 p=0,003	1148±21,8 p<0,001
	50	222±4,40 p<0,001	1210±18,9 p=0,002
	60	150±6,08 p<0,001	1129 [1116; 1156] p<0,001
1/1000 DL50	10	191 [188; 200] p=0,307	963 [945; 980] p=0,199
	20	196 [192; 204] p=0,076	985 [934; 1005] p=0,096
	30	199±8,71 p=0,005	993 [965; 1010] p=0,013
	50	192 [188; 200] p=0,03	978 [956; 1005] p=0,045
	60	197 [190; 210] p=0,016	980 [964; 1005] p=0,140
Control	10	189±5,31	932 [883; 977]
	20	192±5,71	950 [882; 975]
	30	188 [185; 190]	944 [922; 970]
	50	186±3,43	946 [893; 956]
	60	187 [184; 193]	963 [948; 977]

note: p is the level of statistical significance in relation to control

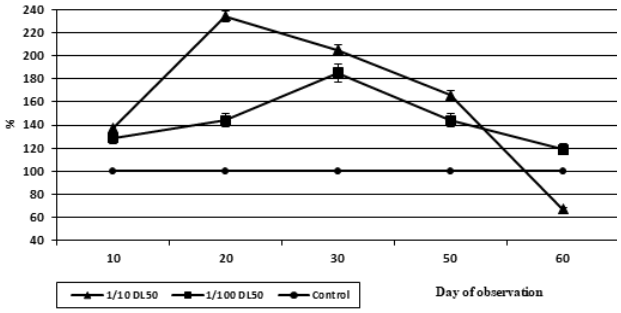

Fig. 1. Dynamics of changes in the intensity of CL in the liver of rats during toxification with sodium fluoride in doses of 1/10 and 1/100 DL50

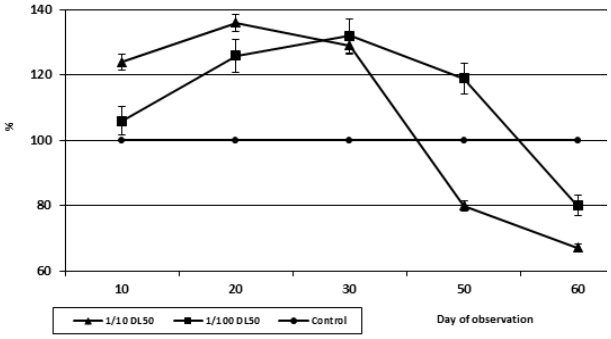

Fig. 2. Dynamics of changes in the activity of NADPH-reductase in microsomes of rat hepatocytes during toxification with sodium fluoride in doses of 1/10 and 1/100 DL50

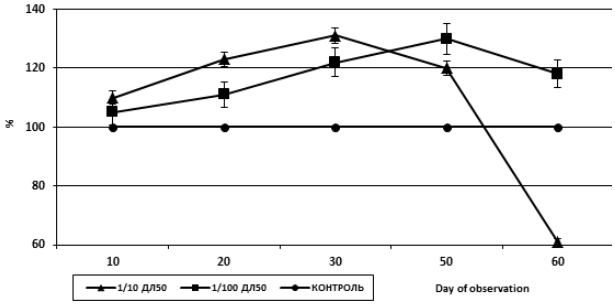

Fig. 3. Dynamics of changes in the activity of NADPH reductase in microsomes of rat hepatocytes during toxification with sodium fluoride in doses of 1/10 and 1/100 DL50

The results obtained indicated a statistically significant (p≤0,002) increase in DC in relation to the control group in all periods of observation. In the case of a dose of 1/10 DL50, the most significant increase in this indicator was observed on the 10th day of the experiment, 265%, and with the introduction of a dose of 1/100 DL50 on the 20th day by an average of 234%. The dynamics of these changes is shown in Fig. 4.

An increase in the amount of TBA-reactans was also observed in the liver of rats of the main group (Table 3). On the 10th day of the experiment, an insignificant increase in the indicator was observed in the main group for both doses (p=0,059 and p=0,199). When a dose of 1/10 DL50 was administered, after 20 days, an increase (p≤0,001) in the level of TBA-reactants in relation to the control group by 27, 41, 78, 133% was clearly determined. The same dynamics was observed when the dose was reduced to 1/100 DL50, at which the increase in the amount of TBA-reactans was 19, 73, 70, 99%, respectively, on days 20, 30, 50, 60 (Fig. 5).

Table 3. Content of lipid peroxidation products in rat liver tissue homogenates under the influence of sodium fluoride in subtoxic doses (n = 10; Me [25%; 75%] or M±s)

Dose	Day of observation	Diene conjugates nM / mg protein	TBA reactants nM / mg protein	Chiff bases mind. units / mg lipids
1/10 DL50	10	9,09±0,88 p<0,001	0,70±0,07p=0,059	0,37 [0,28; 0,40] p=0,545
	20	8,12±0,88 p<0,001	0,91±0,08 p=0,001	0,33 [0,20; 0,47]p=0,290
	30	7,64±0,60 p<0,001	1,04±0,12 p=0,001	1,08 [0,90; 1,15] p<0,001
	50	6,9 [6,2; 7,8] p<0,001	1,45±0,15 p<0,001	1,53±0,16 p<0,001
	60	6,2 [5,9; 7,2] p<0,001	1,76±0,16 p<0,001	1,83 [1,50; 1,91] p<0,001
1/100 DL50	10	7,65 [6,94 8,5] p=0,001	0,66 [0,60; 0,70] p=0,199	0,29±0,07 p=0,791
	20	8,75 [8,54 9,8] p<0,001	0,89 [0,75; 0,94] p=0,019	0,24 [0,20; 0,33] p=0,821
	30	7,35 [6,64 8,0] p<0,001	1,28±0,11p=0,002	0,79±0,15 p<0,001
	50	6,8 [6,0; 7,2] p<0,001	1,38±0,04 p<0,001	0,90±0,12 p<0,001
	60	6,30±0,72 p=0,002	1,49±0,12 p<0,001	0,97±0,09 p<0,001
Control	10	2,49±0,54	0,58 [0,52; 0,69]	0,33 [0,19; 0,50]
	20	2,5 [2,3; 2,8]	0,72±0,13	0,29±0,15
	30	2,9 [2,5; 3,2]	0,75 [0,59; 0,86]	0,39±0,09
	50	2,68±0,58	0,81±0,12	0,33±0,10
	60	3,15 [2,8; 3,5]	0,74 [0,65; 0,88]	0,36±0,07

note: p is the level of statistical significance of significance in relation to control

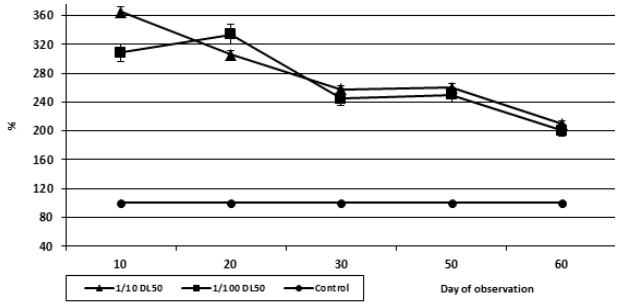

Fig. 4. Dynamics of changes in the content of diene conjugates in rat liver after administration of sodium fluoride at a dose of 1/10 i 1/100 DL50

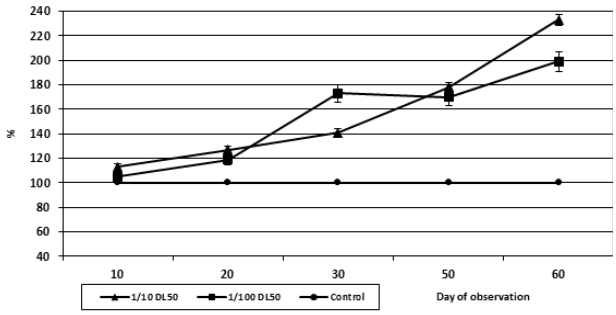

Fig. 5. Dynamics of changes in the content of TBA-reactants in the liver of rats after administration of sodium fluoride at doses of 1/10 and 1/100 DL50

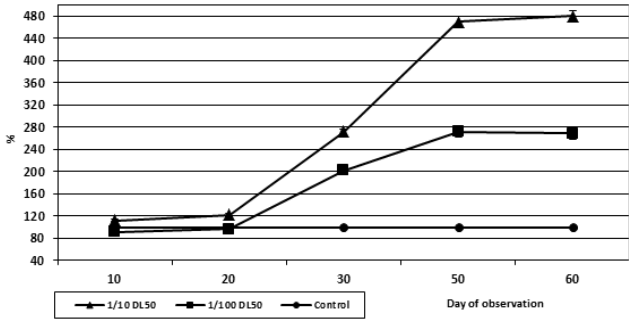

Fig. 6. Dynamics of changes in the content of cipher bases in rat liver after administration of sodium fluoride at a dose of 1/10 i 1/100 DL50

Table 4. Content of cytochromes P-450 and b5 in the microsomal fraction of rat liver under the influence of sodium fluoride in subtoxic doses (n = 10; Me [25%; 75%] or M ± s)

Dose	Day of observation	Cytochrome P-450, nM / mg protein	Cytochrome b5, nM / mg protein
1/10 DL50	10	1,25 [1,18; 1,34] p<0,001	0,93 [0,88; 1,05] p<0,001
	20	1,69±0,09 p<0,001	1,31±0,07 p<0,001
	30	1,32[1,22;1,37] p<0,001	1,21 [1,15; 1,28] p=0,002
	50	0,48±0,16 p<0,001	1,02 [0,85; 1,10]p=0,001
	60	0,40 [0,37; 0,45] p<0,001	0,38 [0,22; 0,49]p<0,001
1/100 DL50	10	1,15±0,07 p=0,004	0,83±0,14 p<0,001
	20	1,36±0,06 p<0,001	1,21±0,07 p<0,001
	30	1,29±0,06 p<0,001	1,36±0,06 p<0,001
	50	1,09 [1,03; 1,11] p=0,011	1,09 [1,08; 1,19] p<0,001
	60	0,54±0,13 p=0,001	0,43 [0,37; 0,52] p<0,001
Control	10	0,88±0,21	0,59 [0,57; 0,60]
	20	0,96 [0,88; 1,12]	0,63 [0,57; 0,68]
	30	1,03 [0,95; 1,18]	0,64±0,11
	50	0,92±0,14	0,69±0,14
	60	0,88±0,19	0,68±0,09

note: p is the level of statistical significance of significance in relation to control

On days 10 and 20, at doses of 1/10 and 1/100 DL50, no statistically important changes in the amount of lipid peroxidation, which are chiff bases, were observed. The results gave an idea of a significant gradual increase (p <0,001) in the level of this indicator at 30, 50, 60 days by 172, 370 and 380%, respectively. A similar, but less pronounced picture was revealed for a dose of 1/100 DL50 - by 103, 173, and 169% (Fig. 6).

Analysis of cytochrome P-450 on days 10, 20, 30 in rats treated with sodium fluoride at a dose of 1/10 DL50 showed a statistically significant (p <0,001) increase in the pool by 44, 74, 23% in comparison with the control group (Table 4).\

In the study of the pool of cytochrome b5 under the influence of sodium fluoride on hepatocytes, it was found to increase by 60, 113 and 78% at a dose of 1/10 DL50. On days 50 and 60,

the level of cytochrome P-450 in microsomes of rat hepatocytes decreased in comparison with the control group (p <0,001) by 48 and 52%, respectively. The level of cytochrome b5 increased on the 50th day by 50% and decreased on the 60th day by 47%.

Oral administration of sodium fluoride at a dose of 1/100 DL50 led to an increase (p≤0,001) in the amount of cytochrome P-450 by day 50 of the experiment (the most pronounced result on day 20 by 40%), as well as cytochrome b5 (maximum on day 30 by 112 %) (Table 4). On the 60th day of observation, the level of cytochromes in the microsomal fraction of rat hepatocytes with the introduction of a dose of sodium fluoride 1/100 DL50 decreased in comparison with the control group (p<0,001) by 35-39%.

The dynamics of changes in the content of microsomal cytochromes as a percentage of the control group of microsomal cytochromes is shown in Fig. 7,8.

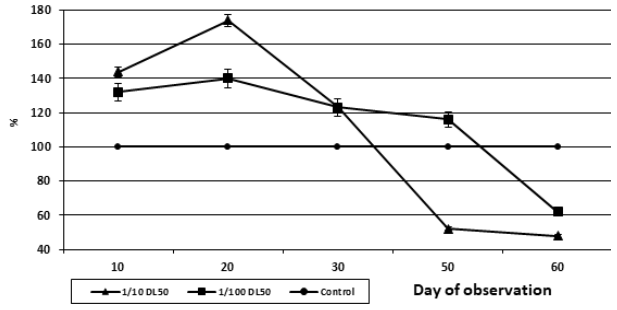

Fig. 7. Dynamics of changes in the total pool of cytochrome P-450 in microsomes of rat hepatocytes after administration of sodium fluoride at doses of 1/10 and 1/100 DL50

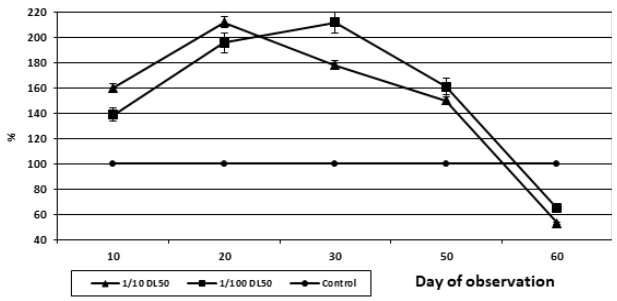

Fig. 8. Dynamics of changes in the content of the total pool of cytochrome b50 in microsomes of rat hepatocytes after administration of sodium fluoride in doses of 1/10 and 1/100 DL50

Conclusions. Long-term oral administration of sodium fluoride, especially at a dose of 1/10 DL50, can lead to a significant disruption of the detoxification function of the microsomal membrane of rat hepatocytes due to the gradual inhibition of the activity of its enzymes and a decrease in the rate of biotransformation of both xenobiotics and endogenous substrates. The increase in the indicators of chiffon bases, dienes and TBA-reactants under the influence of sodium fluoride indicates the purposefulness of the lipid peroxidation process towards the formation of toxic end products.

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SUMMARY

ENZYMATIC ACTIVITY IN MICROSOMES, LIPID PEROXIDATION OF MICE HEPATOCYTES UNDER THE SODIUM FLUORIDE

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Fluorine is one of the most widespread and necessary microelements for the body of animals and humans, which is necessary in a clearly limited amount. Different concentrations of fluorine can affect the state of lipid peroxidation, as well as the functional state of the microsomes of liver hepatocytes. The studies were carried out on mature Wistar rats weighing 180-220 g. Animals were inoculated with an aqueous solution of sodium fluoride once a day for 60 days at doses of 1/10, 1/100 and 1/1000 DL50, which was 20 mg/kg, 2 mg/kg and 0,2 mg/kg body weight. Control rats received drinking water. Each group consisted of 10 animals, the studies of indicators were carried out on days 10, 20, 30, 50 and 60. The induction of free radical processes by sodium fluoride was confirmed using a chemiluminescent reaction of blood serum, the amount of diene conjugates in rat liver tissue homogenates was assessed spectrophotometrically, the content of TBA reactants in rat liver tissue homogenates was determined by the reaction of malondialdehyde and thiobarbituric acid (TBA). The level of chiff bases was determined with a spectrofluorometer, subcellular fractions of the liver were isolated by the method of differentiated centrifugation. An increase in the level of the indicator at a dose of 1/10 and 1/100 DL50 of the intensity of lemiluminescence on the 30th day and its decrease on the 60th day was established.Increase in the activity of NAD (P) H-cytochrome c reductase in the microsomal fraction of the liver at the beginning of the study and a gradual decrease on the 50th and 60th days when using both dosages. The same dynamics was observed for NADH-cytochrome c reductase. With respect to diene conjugates of TBA-reactants and chiff bases, a tendency to increase was observed at all periods of the experiment. Indicators of cytochrome P-450 cytochrome b5 were increased up to 30 days and gradually decreased by 60 days. Long-term administration of sodium fluoride can cause the formation of toxic products and a decrease in the activity of enzymes of the microsomal membrane of hepatocytes.

Keywords: sodium fluoride, hepatocytes, toxic effect, microsomes, lipid peroxidation.

РЕЗЮМЕ

ФЕРМЕНТАТИВНАЯ АКТИВНОСТЬ МИКРОСОМ, ПЕРЕКИСНОГО ОКИСЛЕНИЯ ЛИПИДОВ ГЕПАТОЦИТОВ КРЫС ПОД ДЕЙСТВИЕМ ФТОРИДА НАТРИЯ

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Фтор является одним из значимых для организма животных и человека микроэлементов, который необходим в четко лимитированном количестве. Различные концентрации фтора могут влиять на состояние перекисного окисления липидов и функциональное состояние микросом гепатоцитов печени.

Исследования проведены на половозрелых крысах линии Wistar массой 180-220 г. Животных через зонд затравливали водным раствором фторида натрия, раз в сутки в течение 60 дней в дозах 1/10, 1/100 и 1/1000 $ДЛ_{50}$, что составило 20 мг/кг, 2 мг/кг и 0,2 мг/кг массы. Крысы контрольной группы (n=10) получали питьевую воду. Крысы в зависимости от дозы фторида натрия разделены на группы, по 10 животных в каждой, оценка показателей проводилась на 10, 20, 30, 50 и 60 сутки. Подтверждение индуцирования фторидом натрия свободнорадикальных процессов выполнялось при помощи хемилюминесцентной реакции сыворотки крови, количество диеновых коньюгат в гомогенатах ткани печени крыс оценивали спектрофотометрически, а содержание ТБК-реактантов - реакцией малонового диальдегида и тиобарбитуровой кислоты (ТБК). Уровень шифовых оснований определяли спектрофлюорометрией, субклеточные фракции печени выделялись методом дифференцированного центрифугирования. Установлено повышение уровня показателя в дозе 1/10 и 1/100 $ДЛ_{50}$ интенсивности лемилюминесценции на 30 сутки и его снижение на 60 сутки; повышение активности НАД(Ф) Н-цитохром редуктазы в микросомальной фракции печени вначале исследования и постепенное снижение на 50 и 60 сутки при использовании обеих дозировок. Подобная динамика прослеживалась относительно НАДН-цитохром редуктазы. Что касается диеновых коньюгат, ТБК-реактантов и шифовых оснований наблюдалась тенденция к их увеличению на всех сроках эксперимента. Показатели цитохрома Р-450, цитохрома b5 были повышены до 30 суток и постепенно снижались к 60 суткам. Длительное введение фторида натрия способно вызывать образование токсических продуктов и снижение активности ферментов микросомальной мембраны гепатоцитов.

რეზიუმე

მიკროსომების ფერმენტული აქტივობის და ლიპიდების ზეჟანგური ჟანგვის მდგომარეობა ვირთაგვების ჰეპატოციტებში ნატრიუმის ფთორიდის მოქმედების პირობებში

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ფთორი ადამიანისა და ცხოველის ორგანიზმისათვის წარმოადგენს ერთ-ერთ მნიშვნელოვან მიკროელემენტს, რომელიც მკაცრად ლიმიტირებული ოდენობითაა აუცილებელი. ფთორის სხვადასხვა კონცენტრაციამ შესაძლოა იმოქმედოს ლიპიდების ზეჟანგური ჟანგვის მდგომარეობასა და მიკროსომების ფუნქციურ მდგომარეობაზე ვირთაგვების ჰეპატოციტებში.

კვლევა ჩატარდა Wistar-ის ხაზის, 180-200 გრ მასის ზრდასრულ ვირთაგვებზე. ცხოველებში ზონდით შეიყვანებოდა ნატრიუმის ფთორიდის წყალხსნარი,დღეში

ერთხელ 60 დღის განმავლობაში დოზებით 1/10, 1/100 და 1/1000 $ДЛ_{50}$, რაც შეადგენდა 20 მგ/კგ, 2 მგ/კგ და 0,2 მგ/კგ მასაზე. საკონტროლო ჯგუფის ვირთაგვები (n=10) იღებდნენ სასმელ წყალს. ვირთაგვები, ნატრიუმის ფთორიდის დოზის მიხედვით, დაიყო ჯგუფებად, თითოეულში – 10 ცხოველი; მაჩვენებლების შეფასება ხორციელდებოდა მე-10, მე-20, 30-ე, 50-ე და მე-60 დღეს. თავისუფალრადიკალური პროცესების ინდუცირება ნატრიუმის ფთორიდით დასტურდებოდა სისხლის შრატის ქმილუმინესცენტური რეაქციით, დიენური კონიუგატების რაოდენობა ღვიძლის ქსოვილის ჰომოგენატებში შეფასდა სპექტროფოტომეტრულად, ხოლო თიობარბიტურატმჟავას რეაქტანტებისა - მალონური დიალდეჰიდის და თიობარბიტურატმჟავას რეაქციით. დადგენილია მაჩვენებლის მომატება დოზის 1/10 და 1/100 $ДЛ_{50}$ შემთხვევაში ლუმინესცენციის ინტენსივობის 30-ე დღეს და მისი შემცირება მე-60 დღეს, NAD(P)-ის H-ცოტოქრომის და რედუქტაზას მომატება ღვიძლის მიკროსომულ ფრაქციაში კვლევის დასაწყისში და თანდათანობთი შემცირება 50-ე და მე-60 დღეს ორივე დოზის გამოყენების შემთხვევაში. თიობარბიტურატმჟავას რეაქტანტების დიენური კონიუგატების შემთხვევაში ექსპერიმენტის ყველა ვადაზე აღინიშნა მატების ტენდენცია. ციტოქრომ-P-450 და ციტოქრომ-b5 მომატებული იყო 30-ე დღემდე და შემდეგ თანდათანობით მცირდებოდა 60-ე დღემდე. ნატრიუმის ფთორიდის ხანგრძლივმა შეყვანამ შესაძლოა გამოიწვიოს ტოქსიკური პროდუქტების წარმოქმნა და ჰეპატოციტების მიკროსომული მემბრანის ფერმენტული აქტივობის შემცირება.

A MULTIPLEX PCR ASSAY FOR THE DIFFERENTIAL DETECTION OF *OPISTHORCHIS FELINEUS* AND *METORCHIS BILIS*

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Trematodes are parasites that have caused severe damage to human health since antiquity [1]. There are more than 91 species that infect humans and belong to 46 genera all over the world. According to their habitat in definitive hosts, they are classified as blood flukes, liver flukes, lung flukes, throat fluke, pancreatic fluke, and intestinal flukes [2]. Liver flukes belong to the family of *Opisthorchiidae* include 33 genera cause opisthorchiasisin piscivorous mammals, birds, and humans [3]. Human populations show high levels of infection with the main three liver fluke species within each of their distributional ranges [2]. Up to 680 million people worldwide are at risk of infection [4]. Recent estimates indicate that 45 million people living in Asia and Europe are infected, with approximately 35 million *C. sinensis* cases, 10 million *O. viverrini* cases, and 1.2 million cases of *O. felineus* [5-7]. The pathogen *M. bilis*, which occurs in the same territory as *O. felineus*, has attracted particular attention. It is widely registered in Russia and Kazakhstan, and there are several cases of mixed infections in humans and animals [8-13].

A variety of methods have been established for the effective diagnosis of opisthorchiasis infection, which include antigen-specific enzyme-linked immunosorbent assay (ELISA) [14-16] and various other polymerase chain reaction (PCR) technologies [17-19]. ELISA kits available on the market are not capable of the failure of *O. felineus* and *M. bilis* species detection in opisthorchiasis infection what is one of the major deficiencies. There are no commercially available molecular diagnostic kits for the simultaneous detection of mixed infections by *O. felineus* and *M. bilis*. Therefore, there is no clear understanding of the distribution of each of these species, their localization in the definitive host and approaches to treatment. The aim of this study was, therefore, to establish a multiplex PCR assay for the differential detection of *O. felineus* and *M. bilis* in clinical specimens, which will be necessary for the epidemiology, diagnoses, and control of trematodes infections.The advantage of this method of molecular diagnostics is the high specificity of the reaction, the speed of the results obtained, and the possibility of differential diagnosis of two types of pathogens.

Material and methods. *Samples collection and DNA extraction*

Samples of adult worms of *O. felineus* and *M. bilis* were collected from the artificially infected Syrian hamsters (Akmola region) and infected foxes (Karaganda region) in the territory of Kazakhstan. Genomic DNAs were extracted from adult parasites using the BioSilica DNA extraction kit (Novosibirsk, Russia), according to the manufacturer's instructions. Duodenal bile and feces samples of humans suspected of contracting infectious diseases were kindly provided by Astana Infectious Diseases Hospital, Kazakhstan, in compliance with patient confidentiality, and stored at −80°C until DNA extraction. Sample preparation was carried out according to the method of Duenngai K. et al.: a sample (feces - 500 mg, bile - 0.5 ml) is mixed with 4 ml of physiological saline and 0.4 ml of ethyl acetate, centrifuged at 4000 rpm for 10 min, followed by removal of the supernatant [20]. Genomic DNA was extracted from bile and feces samples using a method recommended by Duenngai K. et al. with some modification. The amount and purity of the extracted DNA could be determined by measuring absorption at 260 nm and 280 nm in the NanoDrop 2000 (Thermo Scientific, USA). DNA was dissolved in ddH_2O and stored at – 70°C.

Standard PCR. Fragments of co1 gene were amplified using primer pair (*OpiOpe2-co1F* 5'-TGGGGAGTTGATTTTTTGATGTT-3' / *COI-uniRv* 5'-AGCAATAACAAATCAAGTATCATG-3') for both opisthorchiids in order to reveal species-specific nucleotide substitutions. The PCR product was sequenced and deposited in GenBank (MT325502 - MT325505).

Species-specific primer design. Based on the *COX1* sequences, genome DNA from *O. felineus* and *M. bilis* were designed the multiplex PCR primers by targeting conserved sequences flanking variable regions with online free available primer programs PerlPrimer v1.1.21 (http://perlprimer.sourceforge.net) and Oligo Analyzer 1.2 software (http://www.genelink.com). Details of primer pairs are presented in Table 1.

Table 1.Forward and reverse primers used in the multiplex PCR for O. felineus and M. bilis

Species	Primers	Sequences 5′-3′	Products (bp)
O. felineus	*CO1nOf-F*: *CO1nOf-R*:	5′-TTGGAATGATTAGTCATGTTTGTACG-3′ 5′-CCCCACCTATAGTAAAAAGCACTAT-3′	307
M. bilis	*COInMb-F*:*COInMb-R*:	5′-TGTTAATATTGCCGGGGTTTG-3′ 5′-TTTATCCCAGTAGGAACACCTATAAC-3′	252

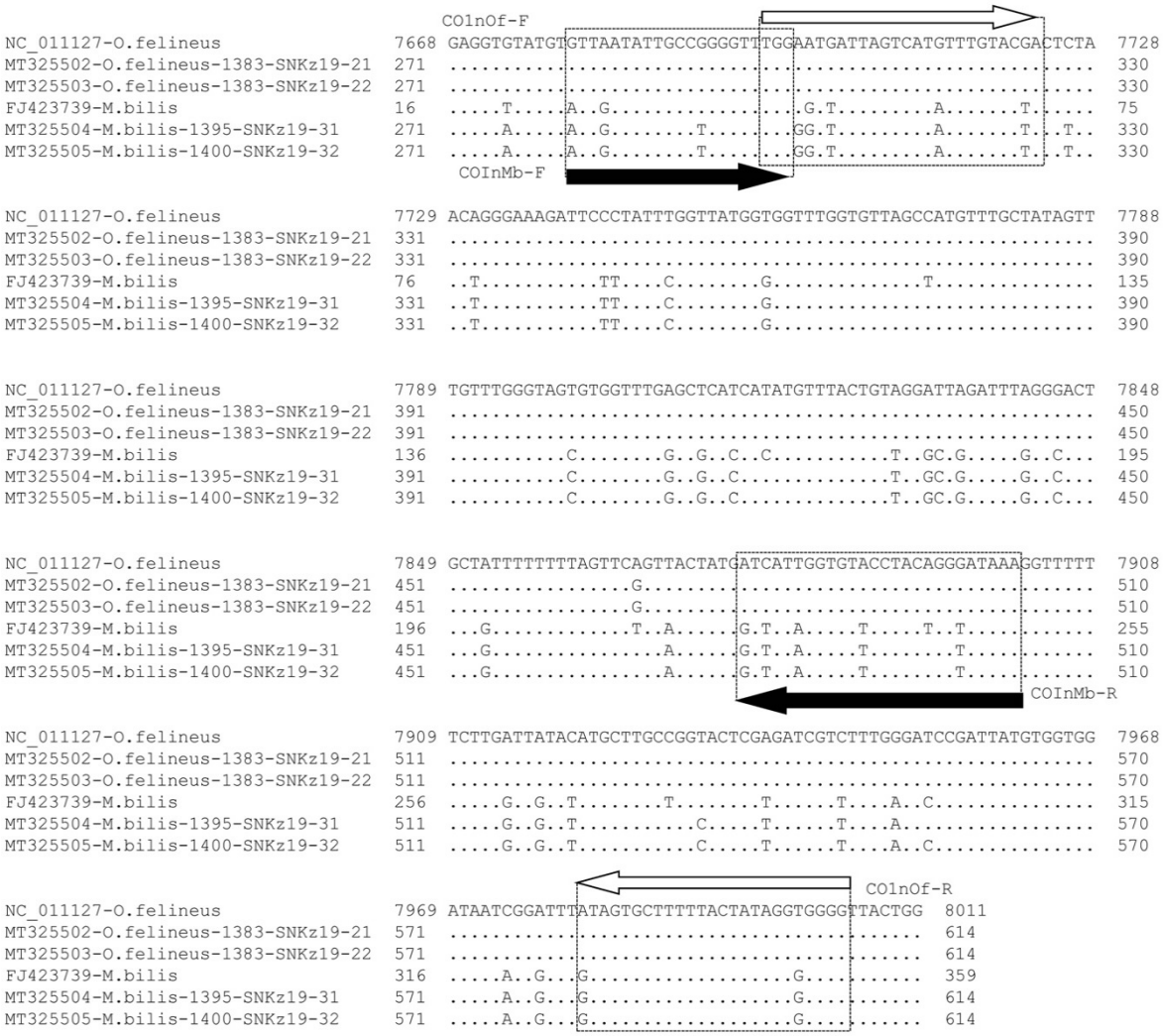

Fig. 1. COX1 fragments alignment used for primers design

Separated PCR and the standard conditions. The PCR parameters were optimized, and the reaction was carried out in a final reaction volume of 25µl containing 1ˣ Hot Start PCR Buffer (20 mM KCl, 5 mM $(NH_4)_2SO_4$, 20 mM Tris-HCl, pH 8.3), 2.5 mM $MgCl_2$ and 1 U Maxima Hot Start Taq polymerase (Thermo Scientific, USA), 2 mM of dNTP, 100 pmol of each *COX1* primer (*CO1nOf-F/R* or *COInMb-F/R*) and 30 ng extracted trematode DNA. In order to detect potential contamination, a PCR mixture with RNA/DNA-free water was regularly used as a negative control. PCR was performed as follows: denaturation at 95°C for 5 min, followed by 35 cycles with 30 s denaturation at 95°C,primer annealing at 59°C for 40 s, and extension at 72°C for 50 s with a final extension during 5 min at 72°C; and amplification products were stored at 4°C until they were visualized. PCR products (7µl) were visualized by electrophoresis in 1.5% agarose gels that were pre-stained with ethidium bromide (EB) and viewed under UV light (VilberLourmat UV-Transilluminator + with Bio-Capt software). The electrophoretic buffer solution was 1ˣTAE buffer.

Optimization of multiplex PCR primers. Optimization of the primer combinations was based on the specificity of the work of primers with DNA pathogens. The amplification of the conserved region of the partial *COX1* gene of *O. felineus* and *M. bilis* using 100 pmol of each specific primer pair (*CO1nOf-F/R* and *COInMb-F/R* primers) was carried out in a 25 µl reaction volume as described above. To examine specificity, the primers were tested together and separately with both DNA from *O. felineus* and *M. bilis*.

Optimization of multiplex PCR conditions. The multiplex PCR reaction is affected by many factors. In order to obtain the best reaction parameters, the multiplex PCR was optimized by varying single parameters while other parameters were maintained. Therefore, the parameters of the PCR assay were optimized by the varying temperature of primers annealing (56, 58, 60, 62, 64, 66°C), the concentration of dNTPs (0.05-0.5 mM) and Taq DNA polymerase

(1.0, 1.5, 2.0, 2.5 and 5.0 U) in a 25-µl reaction volume. A mixture of the genomic DNA, which contained the same amount of genomic DNA of the two types of parasites, was used as a template to amplify the corresponding target genes. The total volume of each reaction system was 25 µl, which included 1 µl of template DNA (about 15 ng of genomic DNA). PCR was performed as follows: denaturation at 95°C for 5 min, followed by 25-40 cycles with 15 s denaturation at 95°C, primer annealing at 62°C for 25 s, and extension at 72°C for 30 s with a final extension during 5 min at 72°C. After the reaction, 7 µl of the reaction solution was mixed with 7 µl of loading buffer for 1.5% agarose gel electrophoresis.

The sensitivity of the multiplex PCR assay. The sensitivity of the multiplex PCR assay was evaluated using a tenfold serial dilution method. The limit of the multiplex PCR assay detected DNA was verified using serial dilutions of mix genomic DNA of each parasite in nuclease-free water, and the final DNA concentration for each parasite in a 25µl reaction system was 10 ng/µl, 1 ng/µl, 100 pg/µl, 10 pg/µl, 1 pg/µl, respectively.

The specificity of the multiplex PCR assay. The specificity of the multiplex PCR was verified using genomic DNAs of other common worms (*Taenia spp.*, *Toxocara spp.* and *Trichinella spp.*) inhabiting the intestine of human and genomic DNAs of main bacteria (*E. coli*, *Pseudomonas spp.* and *Bacillus spp.*) found in the contents of the gallbladder.

Multiplex PCR for the detection of parasites from naturally infected samples. The Multiplex PCR was verified using genomic DNAs from human feces and bile as templates. Clinical samples were collected from the humans suspected of contracting infectious diseases that were kindly provided by Astana Infectious Diseases Hospital, Kazakhstan. The multiplex PCR assay was carried out in a final reaction mixture of 25µl, containing 2µl templates, 4µl optimal primers with 0.5µl from each forward and reverse primer (*CO1nOf-F/R* or *COInMb-F/R*), and 1 µl Maxima Hot Start Taq polymerase (Thermo Scientific, USA), followed by thermal cycling conditions and visualization process mentioned above.

The biosafety ethics for this research was approved by the Animal Ethics Committee of Veterinary Medicine Faculty of KATU (Ethical approval letter, No. 1, 09.11.2017), before commencing the project.

Results and discussion. The amplification of the genome DNA from *O. felineus* and *M. bilis* produced products of 709 bp length. The fragments ~ 345 bp were subjected to sequence analysis to design species-specific primers (Fig. 1).

Four species-specific primers produced the DNA fragments of 307 bp *(O. felineus*) or 252 bp *(M. bilis*) in PCR analysis as expected (Fig. 2).

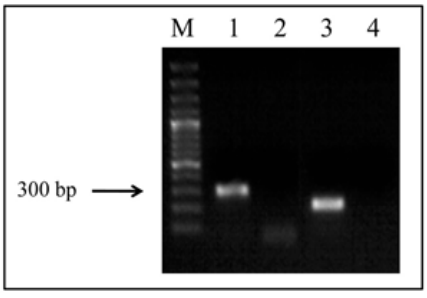

Fig. 2. Electrophoregram of a single PCR under standard conditions: Lane M, DNA ladder (bp); lane 1, O. felineus; lane 3, M. bilis; lane 2, 4, negative control

The multiplex PCR products of mixed templates of the two parasites are shown in Fig. 3. The product containing two DNA bands (307 and 252 bp) was amplified with mixed DNA templates of *O. felineus*/*M. bilis* and each specific primer pair (*CO1nOf-F/R* and *COInMb-F/R*). The results showed that the optimal annealing temperature of the multiplex PCR reaction was 60 to 62°C (Fig. 4), while the optimal dNTP and Taq Polymerase concentrations were 0.3 mM (Fig. 5) and 1.5 U (data not shown), respectively. The number of cycles largely determines the required total duration of the multiplex PCR assay. The optimal number of multiplex PCR cycles was 35, which is the standard number in this reaction (Fig. 6). In addition, the concentrations of each pair of primers were optimized, and the results showed that the optimal concentration of each pair of oligonucleotide primers was 0.1 µM (data not shown).

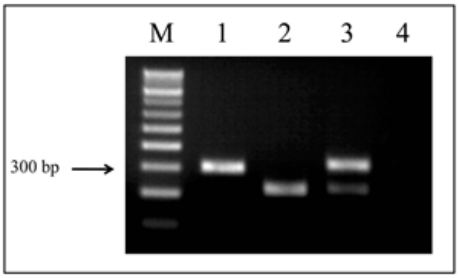

Fig. 3.Electrophoregram of multiplex PCR with both DNA from O. felineus and M. bilis: Lane M, DNA ladder (bp); lane 1, CO1nOf-F/R primers; lane 2, COInMb-F/R primers; lane 3, CO1nOf-F/R and COInMb-F/R primers; lane 4, negative control

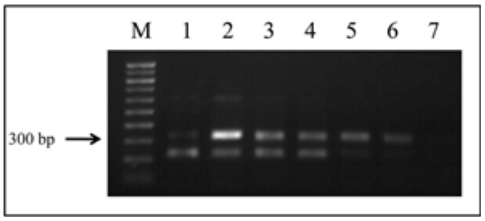

Fig. 4. Electrophoregram of multiplex PCR products in optimization of varying temperature of primers annealing conditions: Lane M, DNA ladder (bp); lane 1, 56°C; lane 2, 58°C; lane 3, 60°C; lane 4, 62°C; lane 5, 64°C; lane 6, 66°C; lane 7, negative control

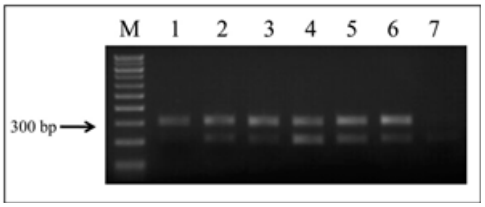

Fig. 5. Electrophoregram of multiplex PCR products in optimization concentration of dNTPs conditions: Lane M, DNA ladder (bp); lane 1, 0.05 mM; lane 2, 0.1 mM; lane 3, 0.2 mM; lane 4, 0.3 mM; lane 5, 0.4 mM; lane 6, 0.5 mM; lane 7, negative control

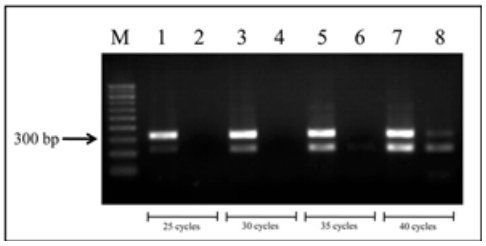

Fig. 6. Electrophoregram of multiplex PCR products in cycles optimization conditions: Lane M, DNA ladder (bp); lane 1, 3, 5, 7, positive control; lane 2, 4, 6, 8, negative control

The sensitivity of the proposed multiplex PCR assay was defined as the minimum DNA molecule concentration, which could be detected. DNA standards, which were diluted from 10 ng to 1 pg, were used for the multiplex PCR. As shown in Fig. 7, the detection limit of the multiplex PCR for *O. felineus* and *M. bilis* was 100 pg, respectively.

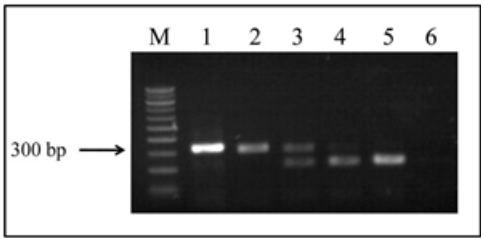

Fig. 7. Determination of the sensitivity of the multiplex PCR. Lane M, DNA ladder (bp); lanes 1-5, the concentration of each O. felineus/M. bilisDNA were 10 ng/1 pg, 1 ng/10 pg, 100 pg/100 pg, 10 pg/1 ng, 1 pg/10 ng, respectively; lane 6, negative control

In order to confirm the specificity of the multiplex PCR developed in this study, the genomes of three species of parasites (including *Taenia spp.*, *Toxocara spp.* and *Trichinella spp.*) and three bacteria (*E. coli*, *Pseudomonas spp.* and *Bacillus spp.*) were selected as the DNA template for reaction under optimized conditions (Fig. 8). The multiplex PCR test was performed with the genomes of *O. felineus* and *M. bilis* as the template DNA, which served as a positive control.

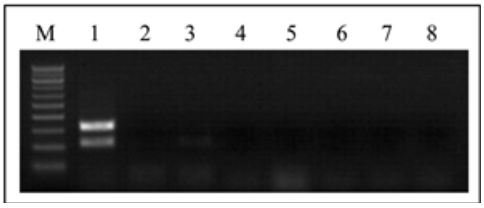

Fig. 8. Test for specificity of the multiplex PCR assay: Lane M, DNA ladder (bp); lane 1, positive control; lane 2, negative control; lane 3, Taenia spp.; lane 4, Toxocara spp.; lane 5, Trichinella spp.; lane 6, E. coli; lane 7, Pseudomonas spp.; lane 8, Bacillus spp

By developed multiplex PCR assay, a total of 9 feces and 2 human bile samples were tested. Four feces samples tested positive with a fragment size of about 252 bp identified as *M. bilis* infection (Fig. 9). One feces sample showed a double band with a fragment size of about 252 and 307 bp featuring a mix of *O. felineus* and *M. bilis* infection. Four feces samples showed a negative result. Two bile samples showed also showed a double band pointing to a mixed of *O. felineus* and *M. bilis* infection.

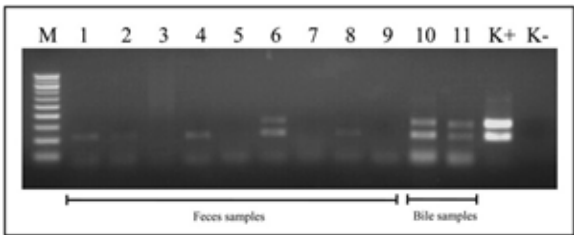

Fig. 9. The PCR results of DNA sample from human feces and bile: Lane M, DNA ladder (bp); lane 1-9, feces samples; lane 9, 10, bile samples; lane K+, positive control; lane K-, negative control

Currently, fecal examination for the detection of parasite eggs and serological studies by ELISA are the main methods for the detection of opisthorchiasis infection. However, these methods of diagnosing have some drawbacks. There are very laborious and fail to accurately distinguish the species of the pathogen [14,21-24].

Molecular methods can be an alternative to the existing methods because they have a high sensitivity and specificity, cheapness during mass screening, as well as the ability to determine the species affiliation at any life stage of the parasite. It is crucial for the sanitary-epidemiological and environmental monitoring. Primer specificity is a critical determinant of the success of a multiplex PCR assay. Pauly A. et al. reported the use of the part of the mitochondrial cytochrome c oxidase I gene for specific detection of adult specimens of the opisthorchiid liver fluke species *O. felineus* and *M. bilis* [17]. Kang S. et al. developed a fast and accurate molecular identification system for human-associated liver fluke species (*Opisthorchis viverrini*, *Opisthorchis felineus*, and *Clonorchis sinensis*) using the PCR-RFLP analysis of the *18S-ITS1-5.8S* nuclear ribosomal DNA region [19]. Brusentsov I. et al. developed multiplex polymerase chain analysis for identification of the ribosomal RNA gene cluster fragment incorporating internal transcribed spacer 2 (*ITS2*) of differentiates parasitic diseases induced by *O. felineus* and *M. bills* [18]. These are all examples of the possible use of PCR methods for the precise identification of parasites, but the methods have not been further developed, have not been commercialized, and have not yet found their practical application.

Mitochondrial genes are amongst the most popular molecular markers that have been widely used in molecular diagnoses of parasitic organisms [17,25,26]. Therefore, we explored the *COX1* genes as molecular markers to develop a multiplex PCR assay for the differential detection of *O. felineus* and *M. bilis* in clinical specimens (Fig. 1). In this study, we developed a multiplex assay that is sensitive to discriminate and diagnose two trematode parasites (*O. felineus* and *M. bilis*) simultaneously in a single reaction compare to the conventional PCR method. The specificity analysis showed that no cross-reactivity was observed between each other (Fig. 3), as well as with other worms inhabiting the small intestine [27] and main bacterial strains found in various pathologies in the bile [28] (Fig. 8).

In most cases, the sensitivity of a multiplex PCR assay will be reduced with increased numbers of target genes in the system. However, the minimum detected DNA of the proposed multiplex PCR assay was 100 pg, low enough to produce results in case of low DNA yield, which is in agreement with the results of previous studies.

For optimization of the multiplex PCR assay, varying temperature of primers annealing, the concentrations of dNTPs and Taq DNA polymerase, and, as well as the optimal number of multiplex PCR cycles, were optimized in this study. The concentrations of the primer pairs for *O. felineus* and *M. bilis* were set at 0.1 μM, and the specificity was judged as appropriate. Experimentally established that the optimal annealing temperature of the multiplex PCR reaction was 60 to 62°C, so in further experiments, we used a temperature of 62°C. dNTPs are raw materials for the synthesis of target fragments. In this study, the target fragments were all 559 bp, but two were synthesized. Therefore, under consideration of obtained results, we recommend a dNTP concentration of 0.3 mM to amplify the corresponding target fragments. Based

on experimental results and cost, the optimal concentration of Taq DNA polymerase was recommending 1.5 U per reaction. The optimal number of multiplex PCR cycles was 35, which allowed producing the required number of amplicons for good visualization of the result in the agarose gel and to avoid a false-positive result.

In addition, to determine whether the multiplex PCR assay was appropriate for the detection of pathogens in clinical samples, 9 feces and 2 human bile samples were tested. The results showed that two pathogens (*O. felineus* and *M. bilis*) were detected in the 1 stool sample and 2 bile samples with the proposed multiplex PCR assay. 4 stool samples showed the presence of the *M. bilis* pathogen. 4 stool samples showed a negative result. Accordingly, the present results indicated that the assay could be used in clinical investigation.

Conclusion. In conclusion, the multiplex PCR assay is an efficient tool for the detection, and simultaneous diagnosis of *O. felineus* and *M. bilis* trematodes from clinical specimens, the lowest limit of detectable DNA was 100 pg for two parasites. The developed method of molecular diagnostics will be used to study the spread of the O. felineus and M. bilis pathogens in humans and animals on the territory of the Republic of Kazakhstan and their role in the etiology of opisthorchiasis infection. Consequently, this essay will be potentially useful in epidemiological studies, diagnosis, and treatment of opisthorchiasis infections.

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SUMMARY

A MULTIPLEX PCR ASSAY FOR THE DIFFERENTIAL DETECTION OF *OPISTHORCHIS FELINEUS* AND *METORCHIS BILIS*

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Opisthorchis felineus and *Metorchis bilis* are two common small worms that parasitize in the gallbladder and bile ducts of the liver of humans and carnivores. These parasites have a severe impact on health and are considered pathogens of serious diseases worldwide, such as cholangiocarcinoma. However, there are still no commercially available molecular diagnostic kits capable of simultaneously detecting these parasites in humans. Therefore, the study aimed to develop a multiplex PCR analysis that will differentially determine these two opisthorchiasis infections in one reaction. Two specific primer pairs for a multiplex polymerase chain reaction (PCR) were designed based on corresponding mitochondrial genome sequences. The multiplex assay detection limit was assessed by serial dilutions of the genomic DNAs of trematode worms examined. Naturally, infected samples of human bile and feces were tested using the developed assay. A multiplex PCR assay was developed based on mitochondrial DNA that accurately and simultaneously identifies two trematode species in one reaction using specific fragment sizes of 307 and 252 bp for *O. felineus* and *M. bilis*, respectively. The optimal reaction conditions, specificity, and sensitivity of the multiplex PCR assay were investigated. The lowest DNA concentration detected was 100 pg for *M. bilis* and *O. felineus* in a 25µl reaction system. This study provides an efficient tool for the simultaneous detection of *O. felineus* and *M. bilis*. The proposed multiplex PCR assay will be potentially useful in epidemiological studies, diagnosis, and treatment of this mixed opisthorchiasis infection.

Keywords: *COX1*, *Metorchis bilis*, *Opisthorchis felineus*, multiplex PCR.

РЕЗЮМЕ

МУЛЬТИПЛЕКСНЫЙ ПЦР-АНАЛИЗ ДЛЯ ДИФФЕРЕНЦИАЛЬНОГО ОБНАРУЖЕНИЯ *OPISTHORCHIS FELINEUS И METORCHIS BILIS*

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Opisthorchis felineus и *Metorchis bilis* - два распространенных небольших червя, которые паразитируют в желчном пузыре и желчных протоках печени человека и плотоядных животных. Эти паразиты считаются возбудителями серьезных заболеваний, таких как холангиокарцинома. Однако по сей день не имеется коммерчески доступных наборов для молекулярной диагностики, способных одновременно обнаруживать этих паразитов у человека.

Целью исследования является разработка мультиплексного ПЦР-анализа, который позволит дифференцировать эти две описторхозные инфекции в одной реакции.

Разработаны две специфические пары праймеров для мультиплексной полимеразной цепной реакции (ПЦР) на основе соответствующих последовательностей митохондриального генома. Предел обнаружения мультиплексного анализа оценивался путем серийных разведений геномной ДНК исследуемых трематод. Инфицированные образцы желчи и кала человека протестированы с помощью разработанного метода анализа. Мультиплексный ПЦР-анализ разработан на основе митохондриальной ДНК, которая точно и одновременно идентифицирует два вида трематод в одной реакции с использованием конкретных размеров фрагментов 307 и 252 п.н. для *O. felineus* и *M. bilis*, соответственно. Исследованы оптимальные условия реакции, специфичность и чувствительность мультиплексного ПЦР-анализа. Самая низкая обнаруженная концентрация ДНК составила 100 пг для *M. bilis* и *O. felineus* в 25 мкл реакционной системы. Проведенное исследование представляет собой эффективный инструмент для одновременного обнаружения *O. felineus* и *M. bilis*. Предлагаемый анализ мультиплексной ПЦР потенциально полезен в эпидемиологических исследованиях, диагностике и лечении смешанной описторхозной инфекции.

რეზიუმე

მულტიპლექსური პჯრ-ანალიზი *OPISTHORCHIS FELINEUS*-ის და *METORCHIS BILIS*-ის დიფერენციული აღმოჩენისათვის

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Opisthorchis felineus და *Metorchis bilis* – გავრცელებული მცირე ზომის ჭიებია, რომელიც პარაზიტობს ადამიანის და ძუძუმწოვარი ცხოველების ნაღვლის ბუშტში და ღვიძლის ნაღვლის სადინარებში. ეს პარაზიტები ითვლება სერიოზული დაავადებების გამომწვევებად, კერძოდ, ქოლანგიოკარცინომის. თუმცა, დღემდე არ მოიპოვება მოლეკულური დიაგნოსტიკის კომერციულად ხელმისაწვდომი ნაკრებები, რომელიც შესაძლებელს გახდიდა ამ პარაზიტების ერთდროულად აღმოჩენას ადამიანის ორგანიზმში.

კვლევის მიზანს წარმოადგენდა მულტიპლექსური პჯრ-ანალიზის შემუშავება, რომელიც შესაძლებელს გახდის ამ ორი ოპისტორქოზული ინფექციის დიფერენცირებას ერთი რეაქციის ფარგლებში.

შესაბამისი მიტოქონდრიული გენომის თანმიმდევრობათა საფუძველზე შემუშავებულია პრაიმერების ორი სპეციფიკური წყვილი მულტიპლექსური პჯრ-სთის. მულტიპლექსური ანალიზის აღმოჩენის ზღვარი ფასდებოდა საკვლევი ტრემატოდების გენომური რნმ-ის სერიული განზავებით. ადამიანის ნაღვლის და განავალის იდენტიფიცირებული ნიმუშების ტესტირება განხორციელდა ანალიზის შემუშავებული მეთოდის გამოყენებით. მულტიპლექსური პჯრ-ანალიზი შემუშავებულია მიტოქონდრიული დნმ-ის საფუძველზე, რომელიც ზუსტად და ერთდროულად ახდენს ორივე სახის ტრემატოდის იდენტიფიცირებას ერთი რეაქციის ფარგლებში *O. feline*-ისა და *M. bili*-სთვის კონკრეტული ზომის ფრაგმენტების გამოყენებით, შესაბამისად, 307 და 252 პგ. შესწავლილია მულტიპლექსური პჯრ-ანალიზის რეაქციის ოპტიმალური პირობები, სპეციფიკურობა და მგრძნობელობა. დნმ-ის ყველაზე დაბალმა აღმოჩენილმა კონცენტრაციამ რეაქციული სისტემისათვის შეადგინა 100 პგ *M. bili*-სთვის და 25 მკლ *O. feline*-სათვის. აღწერილი კვლევა წარმოადგენს ეფექტურ ინსტრუმენტს *O. feline*-ის და *M. bili*-ის ერთდროული აღმოჩენისათვის. შემოთავაზებული მულტიპლექსური პჯრ-ანალიზი პოტენციურად სასარგებლოა შერეული ოპისტორქოზული ინფექციის ეპიდემიოლოგიური კვლევის, დიაგნოსტიკისა და მკურნალობისათვის.

BIOLOGICAL CHARACTERIZATION OF BACTERIOPHAGES AGAINST STREPTOCOCCUS AGALACTIAE

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Group B Streptococcus (GBS) or *Streptococcus agalactiae* is a Gram-positive, beta-hemolytic, catalase-negative, and facultative anaerobe coccus, which colonize the gastrointestinal and genitourinary tract [1]. GBS species are sub-classified into ten serotypes depending on the immunologic reactivity of their polysaccharide capsule [2]. *Streptococcus agalactiae* causes serious infection diseases and mostly affects immunocompromised patients with chronic diseases and newborns. Infants can be infected during birth from GBS carrier mother, either intra utero or during birth rupture of membranes, also through the inhalation or swallow of bacteria during the delivery [3]. Currently, available GBS prevention strategies which are given by CDC and its mandatory in Georgia as well will not prevent all cases of early-onset disease. *Streptococcus agalactiae* neonatal sepsis risk factors are bacterial colonization; premature birth, low weight, membrane rapture; high temperature during labor, long dry period, Urinary tract infection, etc. GBS can cause infections such as sepsis, pneumonia, and meningitis. A small number of newborns recovered from GBS infection have a long-term disability [3,4].

Antibiotics and especially Penicillin played important role in GBS prevention and treatment. But for treatment dramatically increase number of penicillin-allergic patients' antibiotic prophylaxis should be done very carefully and determine the penicillin-allergy status of all patients. Erythromycin, Vancomycin, and Clindamycin are recommended for penicillin-allergic individuals [5,6].

Uncontrolled use of antibiotics and increase number of antibiotic resistance strain renewed the interest of the modern world to the alternative antimicrobial agents as are Bacteriophages.

Phages are bacterial viruses that lyse only pathogenic bacteria and cause no damage to the normal microflora. They are non-toxic, cause no allergy, are very specific and the use of phage cocktail composition reduces resistance which gives them the ability to use against antibiotic-resistant strains [7].

Based on above mentioned and great interest of understanding phage potential against Streptococcus agalactiae pathogenic bacterial strains our main goal was to identify and study phages active against GBS.

Material and methods. Between March 2019 and March 2020, 257 anovaginal swabs were collected at "Nia Oniashvili Clinic" Tbilisi, Georgia, and Clinic "Curatio". All specimens were collected using sterile cotton swabs that were submerged in Todd-Hewitt Broth supplemented with colistin and nalidixic acid (Liofilchem, Italy). The samples were cultured following the CDC recommendations [8]. THB was inoculated with the swab and incubated aerobically at 36(±1)°C for 18-24 hours. After incubation, each sample was sub-cultured on Strep B select (Biorad, France) and Columbia with 5% sheep blood agar (BA) plates (Eliava mediaproduction, Georgia). Plates were incubated at 36 (±1)°C for 24 hours aerobically and after incubation was examined.

The presumptive positive samples then were confirmed by biochemical test Strepto Integral System (Liofilchem, Italy), which is a 24-well system containing a desiccated biochemical substrate for the identification and susceptibility testing of streptococci. The system is inoculated with the bacterial suspension of the microorganism to be examined and incubated at 36±1°C for 18-24 hours. At the end of the incubation period, the color change of the various wells allows identifying the organism by its numerical profile.

Serotyping by latex agglutination. All positive samples were serotyped by Strepto B latex Kit (Liofilchem, Italy). Latex particles are individually sensitized with rabbit antibodies specific to Streptococcal antigens of groups B. Bacterial colonies are incubated in an enzymatic suspension to extract antigen. The extract enzyme preparation is tested on a reaction card. In the presence of homologous antigen, particles will aggregate to deliver visible agglutination.

Isolation and purification of bacteriophages: Bacteriophages were isolated from the sewage water of Mtkvari River, Tbilisi, Georgia. 40 mL of wastewater was enriched with 10mL 10x modified TSB (supplemented with 1,2gr $CaCl_2$ per liter and 5mM $MgSO_4$) and added 0,5mL overnight culture of target Streptococcus agalactia. Tubes were incubated at 35^0 C for 24 hours and after incubation was centrifuged at 5000x g for 30min. The supernatant, putative phage lysate was filtered through the 0,22 µm. These phage lysates were screen by spot test assay [9].

Phage host range was determined by spot test serial dilutions of high-titer phage lysates onto lawns of the bacteria strains. Following 24 h of incubation at 35 °C, plates were checked for plaques formation. Lytic activity of phages was designated as CL- confluent lysis; SCL- semi-confluent lysis; IP- individual plaques; OL- overgrown lysis; R- resistant [10].

Electron microscopy. Phages were centrifuged and washed with 0.1 mol/L ammonium acetate.. Negative staining was performed by placing grids in 1% uranyl acetate solution for 2 min. Electron micrographs were taken with a JEM 1400 transmission electron microscope (JEOL) at 100 kV [11].

Phage adsorption. We used a modified Stent's procedure (Stent 1963) where was combined phage $1x10^7$ and bacterial cultures $1x10^8$. Tubes were then placed in a water bath at 35 °C. After 0,3, 5, 7, and 10 min, 0.1 mL samples were taken and transferred into tubes with 9.9 mL of modified TSB supplemented with 0.4 mL of chloroform. The mixture in the tubes was actively shaken then placed on an ice bath for 10 min. Subsequently, each sample was diluted 100-fold, and prepare to 1 mL phage and 0.1ml culture solution mixed with semi-solid TSA and poured over modified TSA plates. Followed by 24 h incubation at 35^0 C, the titer of unattached phages was determined by the special formula: 100-(Pn/P0 x 100). Where, Pn- number of non-adsorbed phages, P0 – phage number in control. Serial dilution of phages without chloroform treatment was used as a control. Attachment assays for each phage were replicated 3 times [11].

DNA extraction and restriction. Phages DNA was isolated by QIAamp DNA Mini Kit (Qiagen) according to the manufacturer's instructions. Purified phage DNA was digested with NcoI, NdeI BamHI, HindIII, KpnI, EcoRI, SpeI, SmaI according to the manufacturer's protocol with some modifications: 10 µl phage genomic DNA was digested for up to 24 h at an appropriate temperature for each restriction endonuclease with 1 µl of a single restriction endonuclease plus 2 µl restriction buffer. Digested DNA was electrophoresed in 1% agarose gels. For the estimation of the size of the DNA fragments, λ genomic DNA digested with HindIII was used.

Results and discussion. The culture identification procedures and preparation revealed that from the obtained 257 swabs 87 were positive for Streptococcus agalactiae. Susceptibility to antibiotics was detected against 12 antimicrobials. All strains were 100% susceptible to Penicillin, Ampicillin, and Vancomycin. However resistant strains to Erythromycin and Clindamycin were observed in 25% and 13% respectively [4]. For phage isolation, we sub-grouped bacterial strains and used different combinations for wastewater sample enrichment procedures. We isolated 2 bacteriophages, which then were designated as vB_GBS_1 and vB_GBS_2 but before the further study, we performed phage purification procedure. Single phage colonies were taken from the plate by puncturing the agar and transformed into the tube with 1mL modified TSB and incubated at 35^0 C for 1 hour. Suspension then was centrifuged at 5000x g for 20min, filtrated through 0,22µm membrane filter, and tittered by double agar layer method. These procedures were repeated 5 times, both phages showed small clear negative colonies on TSA plates (Fig. 1, 2).

Phage colonies.

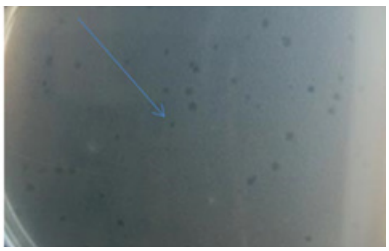
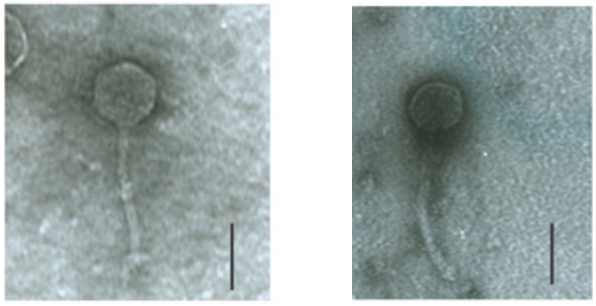


Fig. 1. vB_GBS_1

Fig. 2. vB_GBS_2



A *B*

Fig. 3. A - phage vB_GBS_1 had a 50 nm diameter icosahedral head and a 112 nm tail.
B - phage vB_GBS_2 had a 42 nm diameter head and 120 nm tail

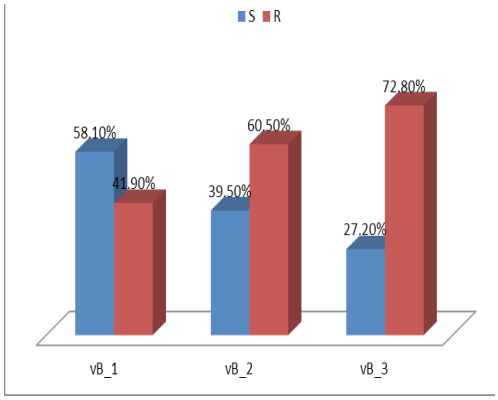


Fig. 4. Phage sensitivity

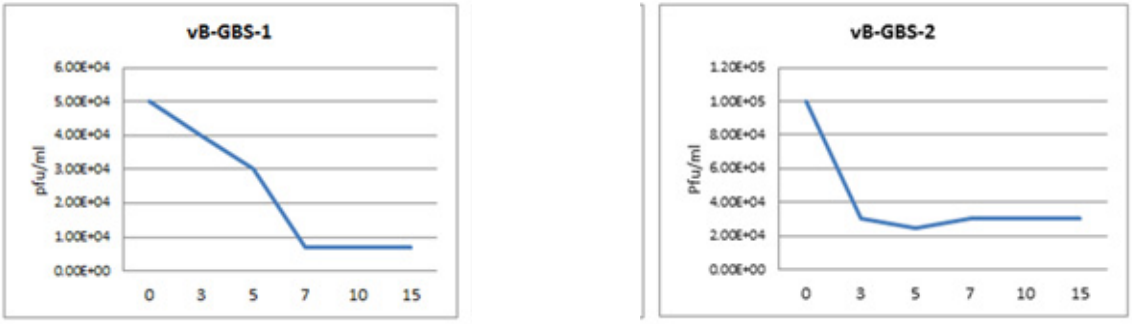


Fig. 5. Phage adsorption

Electron microscopy revealed that both phages belong to the *Syphoviridae* family, Phage vB_GBS_1 had a 50 nm diameter icosahedral head and a 112 nm tail (Fig. 3A). Phage vB_GBS_2 had a 42 nm diameter head and 120nm tail (Fig. 3B).

Transmission electron micrographs of phages stained with 1% uranyl acetate and observed at 100 kV. Magnification is 250 000×. Bars = 50 nm.

Serial dilutions of phages vB_GBS_1 and vB_GBS_2 were spot tested onto lawns of the 87 *Streptococcus agalactiae*, 5 *Streptococcus pyogenes*, 5 *Streptococcus pneumoniae*, 5 *Enterococcus faecalis*, and 5 *Enterococcus faecium* bacterial strains. Phage vB_GBS_1 was able to productively infect 58.1% of *Streptococcus agalactiae* strains. Phage vB_GBS_2 was able to productively infect 39.5% of strains but none of the other species in our collection (Fig. 4).

The time for absorption 85%-90% of each phage was detected to their respective host and for phage vB_GBS_1 it was approximately 7min and 3 min for vB_GBS_2. (Fig. 5).

DNA of Phage vB_GBS_1 was digested only with restriction endonuclease SpeI, DNA of phage vB_GBS_2 shows resistance to all used restriction endonucleases (data not shown).

Heavy genital tract colonization with *Streptococcus agalactiae* is a marker for diagnosis, but even during the active antibiotic therapy antibiotics can't eliminate GBS from the genitourinary and gastrointestinal tracts, and recolonization after a course of antibiotics is typical. In parallel, the number of penicillin allergy is increasing all over the world and it's one of the important problems in antibiotic therapy. The number of Erythromycin - resistant *Str. agalactieae* also increased worldwide. According to this Bacteriophages can lay an important role against *Streptococcus agalactiae* prevention and treatment. Broad-spectrum virulent phages can solve this problem.

Antibiotic resistance of pathogenic bacteria is a major public health problem. On contrary, the development of new antibiotic drugs against resistant strains increases resistance and all-new suggested replacement therapies are more costly and more toxic for human cells. Bacteriophages are biological natural antibacterials that have several advantages over antibiotics and phage–resistant mutants are less virulent as phage receptors are mainly associated with bacterial pathogenicity (12). During the replication process, they have invented many mechanisms to subvert their bacterial hosts.

We isolated and characterized the biological properties of 2 novel phages active against *Streptococcus agalactiae* strains: vB_GBS_1 and vB_GBS_2. Host range experiments showed that both phage types are very specific in lysing the respective *Streptococcus agalactiae* strains. They show different host ranges and restriction profiles. The specificity of these phages ensures that they will not affect native bacterial flora while targeting the host of interest when antibiotics typically affect a broad range of bacterial species, including those that may be beneficial. It would be very interesting to determine the genomic difference of this phages-based sequence and study their lytic cycle to study the therapeutic potential of these phages.

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SUMMARY

BIOLOGICAL CHARACTERIZATION OF BACTERIOPHAGES AGAINST *STREPTOCOCCUS AGALACTIAE*

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Streptococcus agalactiae, also known as group B streptococci, was first isolated from cow's milk with mastitis, and it was first identified in 1930 by Rebecca Lancefield. GBS or streptococcus agalacticae is a gram-positive cocci, beta-hemolytic, facultative anaerobic, which is a harmless inhabitant of the normal human microflora. About 30% of the population is an asymptomatic carrier of this microbe. However, it was considered the leading cause of neonatal invasions upon its discovery. GBS is encapsulated in a polysaccharide capsule, which is a major virulence factor, and 10 serotypes of group B streptococci are known for their immunological activity. According to the existing studies, vaginal colonization is most often caused by type Ia, III, and V in other countries of the world. Today number of invasive GBS diseases continues to increase and it remains a significant pathogen among both infants and adults. The use of broad-spectrum antibiotics has certainly played a major role in the fight against infections, although the number of antibiotic-resistant strains has increased and thus modern medicine starts new insights into alternative antibacterials. Bacteriophages are often suggested as an alternative therapeutic agent against bacterial infections.

In the present study, our aim was to isolate and study bacteriophages active against group B streptococci.

Between March 2019 and March 2020, 257 anovaginal swabs were collected at "Nia Oniashvili Clinic" Tbilisi, Georgia, and Clinic "Curatio"by using standard bacterial identification procedures, in particular group B streptococcus identification guideline provided by CDC we identified 87 Streptococcus agalactiae strains. We used both standard and modified methods to isolate bacteriophages and study their life cycle, which were developed by the team. In this study, two bacteriophages active against Streptococcus agalactiae were identified: *vB_GBS_1 (Syphoviridae)* and *vB_GBS_2 (Syphoviridae)*. The biological characteristics, morphology, adsorption, and host range were studied.

Keywords: Streptococcus agalactiae, antibiotic resistance, Bacteriophages.

РЕЗЮМЕ

БАКТЕРИОФАГИ, АКТИВНЫЕ В ОТНОШЕНИИ СТРЕПТОКОККОВ ГРУППЫ В

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В последнее время *Streptococcus agalactiae*, известный как стрептококк группы В, является одним из значимых патогенов как у новорожденных, так и у взрослых, что связано с постоянным ростом числа антибиотикорезистентных штаммов этих бактерий. Исходя из этого, особое значение приобретают поиски альтернативных антибактериальных средств. Часто в качестве альтернативы антибиотикам для борьбы с инфекциями, вызванными антибиотикорезистентными бактериями, рассматриваются бактериофаги.

Целью исследования явилось выделение и изучение бактериофагов, активных в отношении стрептококков группы В.

В период с марта 2019 г. по март 2020 г. в клиниках «Ниа Ониашвили» и «Куратио» забрано 257 ановагинальных мазков с использованием стандартных процедур идентификации бактерий, в частности руководства по идентификации стрептококков группы В, предоставленного Центром по контролю за заболеваниями. Идентифицировано 87 штаммов *Streptococcus agalactiae*. Для выделения и определения жизненного цикла бактериофагов использованы как стандартные, так и модифицированные нами методы. В пределах проекта выделены два бактериофага: vB_GBS_1 (Syphoviridae) и vB_GBS_2 (Syphoviridae), активные в отношении *Streptococcus agalactiae*. Изучены основные биологические параметры данных фагов – морфология вирионов, адсорбция и диапазон действия.

რეზიუმე

B ჯგუფის სტრეპტოკოკის მიმართ აქტიური ბაქტერიოფაგები

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სადღეისოდ B ჯგუფის სტრეპტოკოკი მნიშვნელოვან პათოგენად რჩება როგორც ახალშობილებში, ასევე მოზრდილებში, რაც დაკავშირებულია ამ სახეობის მიკრობული შტამების მუდმივად მზარდ ანტიბიოტიკორეზისტენტობასთან. აქედან გამომდინარე, თანამედროვე ეტაპზე მიმდინარეობს ანტიბიოტიკორეზისტენტული შტამებით გამოწვეული ინფექციების ალტერნატული სამკურნალო საშუალებების ძიება და ერთ-ერთ საშუალებად განიხილება ბაქტერიოფაგი.

კვლევის მიზანს წარმოადგენდა B ჯგუფის სტრეპტოკოკის მიმართ აქტიური ბაქტერიოფაგების გამოყოფა და შესწავლა. 2019 წლის მარტიდან 2020 წლის მარტამდე, „ნია ონიაშვილის“ კლინიკა და კლინიკა „კურაციო“-დან მიღებულია 257 ანოვაგინალური ნაცხი, ბაქტერიული იდენტიფიკაციის სტანდარტული პროცედურების გამოყენებით, კერძოდ კი B ჯგუფის სტრეპტოკოკის იდენტიფიკაციისთვის გამოყენებული იყო დაავადებათა კონტროლის ცენტრის მიერ შემუშავებული სქემა. სტრეპტოკოკის იდენტიფიკაციის გეგმის გამოყენებით იდენტიფიცირებული იყო Streptococcus agalactiae-ს 87 შტამი. ბაქტერიოფაგების გამოსაყოფად და მათი სასიცოცხლო ციკლის შესასწავლად გამოყენებული იყო როგორც სტანდარტული, ასევე მოდიფიცირებული მეთოდები, რომლებიც შემუშავებული იყო ჩვენს მიერ. კვლევის დროს გამოყოფილი იყო Streptococcus agalactiae-ს მიმართ აქტიური ორი ბაქტერიოფაგი vB_GBS_1 (Syphoviridae) და vB_GBS_2 (Syphoviridae). შესწავლილია მოცემული ფაგების ძირითადი ბიოლოგიური მახასიათებლები - ვირიონის მორფოლოგია, ადსორბცია და მოქმედების დიაპაზონი.

PROVISION OF THE RIGHT TO NON-INTERFERENCE WITH PRIVACY DURING MUSTER PROCESS WITH THE PARTICIPATION OF DOCTOR (FORENSIC EXPERT)

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The current COVID-19 pandemic is creating extreme constraints on health care systems in all member States. The increasing number of severely ill patients raises major ethical challenges that professionals and competent authorities have to address. Difficult decisions have to be taken concerning the society as a collective, and within the health care at an individual level. It is essential that such decisions meet the fundamental requirement of respect for human dignity and that human rights are upheld to ensure that these situations do not increase existing vulnerabilities and do not lead to discrimination in the access to healthcare [17].

According to Dr. Olga Haiub-Kowalczyk “Nobody needs to be convinced of the direct impact on human rights flowing from the pandemic induced by the SARS-CoV-2 virus. The necessity of reorganizing the state and way it works goes hand in hand with sudden changes in how entire societies live, as well as the necessity of adapting to dynamically changing conditions” [22]. Today in many countries of the world the problem of provision of the right to non-interference into privacy is one of the most acute.

The right to respect for privacy is a fundamental human right and a part of the right of privacy, which also includes the right to respect for family life, housing and correspondence. Among the current international legal acts in the sphere of observance of human rights and freedoms, which include non-interference into personal life where Ukraine being a member of, Universal Declaration of Human Rights 1948 [3] should be mentioned; as well as the Article #12, which proclaimed the right to protection from unreasonable interference into privacy. Guarantees of protection of these right are enshrined in Article #8 of the Convention for the Protection of Human Rights and Fundamental Freedoms 1950 (hereinafter – the Convention) [5], and in the Article #17 of the International Covenant on Civil and Political Rights, 1966 [6]. In particular, the Convention provides comprehensive list of cases on interference within privacy by public authorities, which exclude arbitrariness and unlawfulness of such interference. These include the following cases: 1) when the interference is carried out in accordance with the Law; 2) when interference is necessary within democratic society; 3) when the interference is carried out in the interests of national and public security or the economic well-being of the country, for the prevention of riots or crimes, for the protection of health and morality or for the protection of the rights and freedoms of other citizens [5]. The European Court of Human Rights (hereinafter – ECHR) provides guidance on the importance and the range of rights to respect for personal (private) life, which is the source of law in Ukraine [17].

The constitutional and legislative regulation in Ukraine of the right to non-interference with privacy is harmonized with international legal acts. Indeed, Article #32 of the Constitution of Ukraine provides negative obligation of the state to

guarantee the right to non-interference within one's private life, except the cases directly provided by the Basic Law. The provisions of part two of Article #32 of the Constitution of Ukraine acknowledge comprehensive grounds for possible legitimate interference into personal life of a citizen (including one who holds the position related to the functions of the state or local self-government bodies as well as members of his family) [10]. However, the legislation of Ukraine cites a little bit different wording of the mentioned statement than international acts grounded on that possible legitimate interference, which includes the following: 1) consent of a person for collecting, storage, and usage as well as spreading confidential information about him/her; 2) in the way of absence of this consent, there are cases determined by the law and acting in the interests of national security, economic well-being and human rights only. During the pre-trial investigation and consideration them as the matter of fact, every person is guaranteed his/her non-interference of their privacy (personal and family ones) according to Article #15 of the Criminal Procedure Code (hereinafter – the CPC) of Ukraine as the basic document of criminal proceedings.

Despite the updating of the criminal procedural legislation of Ukraine, the legal regulation of the procedure for proceeding individual investigative (searching) actions remains imperfect. In particular, this is directly relevant to the muster process, for which the purposes and targets are not clearly stated; the types of exploration and the procedural process for their implementation are defined insufficiently; the actual reasons for its conduct are not clearly stated in the law; there is no procedural regulation of the process of compulsory mustering. This enables pre-trial investigation bodies to interpret discretion for some provisions of the law on the their own level, which creates conditions for unreasonable restriction of the rights and freedoms of the participants within criminal proceedings involved into conducting investigative (searching) actions [13]. Keeping in mind the mentioned above, the effectiveness for muster proceedings as means of gathering evidence serves as a quick, complete and impartial investigation of crimes; it is important to confirm the guarantees of human rights and freedoms legally, including the right to non-interference within one's personal life, during its procedural conduct. In our opinion, special attention is needed to the legal regulation of cases for obligatory muster process.

The main goal of this article research is to investigate the essence of the concept of the right to non-interference within privacy and to highlight the problems of its secure admission, development of proposals to improve the legislation of Ukraine.

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.

According to Dr. Olga Haiub-Kowalczyk "Despite the seemingly laconic nature of Article 8(1), through dynamic interpretation the right to privacy has become the basis for the discovery of a number of "new" human rights not directly expressed in legal acts, but widely discussed in the doctrine and case law, such as reproductive rights …, the right to knowledge of biological origin …, the right to reputation …, or a number of guarantees for the protection of an individual's personal data. Despite the lack of a coherent model of privacy protection in Europe, there is a common tendency for European countries to take a liberal approach to the right to privacy by guaranteeing individuals new areas of autonomy, in gaining independence from state structures. …The current situation engulfing the world leads us to reflect on the sudden but unnoticed paradigm shift in the contemporary right to privacy. We must accept limitations on it, a return to its roots…" [22]. In order to ascertain the completeness and appropriateness of guaranteeing the right of privacy in the course of mustering, we propose to take the nature and constituent elements of this concept under consideration.

In English language, all aspects of a person's private (personal) life are denoted by the term «privacy», which in literal translation means «lonesomeness», «solitude», «privity», «secrecy» [7]. The private is something that is hidden/enclosed from outside, from somebody's eyes and ears. In this case, for example, personal information, personal data about an individual who is "given an assessment of the nature, appearance, health, material, marital status, lifestyle, personal facts on the biography, as well as the relationships of this individual with relatives, friends, acquaintances, etc." [15]. The word «privacy» in the system of Ukrainian legal terminology is the result of the translation of the English term «privacy», which is used in the sources of law in English-speaking countries. Therefore, it is necessary to define the concept of privacy based on the interpretations presented in the English-language literature. «Private», according to the Great Interpretive Dictionary of Modern Ukrainian, means: «1. Belonging to the individual (s); not state, not public. 2. Concerning the individual (s); personal.// mostly in conjunction with the word life. Not affiliated with the service or social activity, which has no official meaning: non-business (meaning conversation, letter, etc.) [2].

Taking under study the practice of the ECHR, there it can be noticed that the term «privacy» in Article #8 of the Convention has not been disclosed, because it is «a broad-meaning term which cannot be defined thoroughly " (ECHR court case of Peck vs. the United Kingdom (2003)). The concept of "private" life belongs to the sphere of direct personal autonomy. These include aspects of physical and moral integrity. The concept goes beyond the limits of the guarantees of somebody's life free from unwanted publicity [18]. At the same time, The ECHR broadly interprets the concept of "private life", enabling it to respond flexibly and resilience to new issues related to the protection of the right of privacy, which may be derived from new achievements in technology, politics or public life [19].

In particular it should be noted that in the international and national jurisprudence, the terms "private life" and "personal life" are used as synonyms, so in the article as follows the authors will apply to a similar approach.

In order to outline the sphere of realization and guarantee of the right to privacy, it is advisable to mention its components, developed by R. O. Stefanchuk: the ability to have one's private life, i. e. the ability of a person to be the bearer of this personal non-material goodness and to get it in terms prescribed by law; an opportunity to define own private sphere of life independently. This opportunity is characterized by the fact that the law does not provide a list of possible or necessary actions that an individual could exercise his right to determine his personal life, that is the ability to decide independently, at his own discretion, how to define, organize and conduct his personal life, depending on own interests and purposes; possibility of getting acquainted other persons with the circumstances of somebody's personal (private) life, that is the person determines independently the circle of those persons who may have information about his/her personal life; the ability to allow or deny interference with one's private life. This means that the individual determines the possibility of outside personalities to interfere in their privacy or not independently. However, it should be noted that in cases

directly provided by law, in the interests of national security, economic well-being and human rights, this personal right may be restricted; the ability to keep confidential the circumstances of his or her private life means that the individual has the right not to disclose the circumstances of his personal life on his own, as well as to demand such a non-disclosure from other persons who possess this kind of information; the ability to demand protection of the right to privacy, that is the ability to use general and special means of protection in case of violation of the right for privacy [11].

Based on the fact that the ECHR interpreted Article #8 of the Convention in such a way that it prohibits unjustified interference with privacy and the positive obligation to protect against the interference of others, we propose to consider the safeguards for the protection of this right, regulated by the Article #241 "Personality Muster" of the CPC of Ukraine to identify deficiencies and gaps.

Applying the algorithm proposed by the lawyer at the Registry of the European Court of Human Rights D. Vitkauskas to decide whether the right to non-interference within privacy was violated under § 1, Article #8 of the Convention [9], we begin by finding out whether there is an interference within "private" life in the case of examination muster. The answer is going to be "yes" along with consideration of the following.

First of all taking into account the foremost interpretation of the right to privacy in accordance to the ECHR, the fundamental components of modern understanding of the right to privacy are as follows: the origin, surname, first name, health status [20], etc., and also "preservation of one's mental and physical integrity". It goes about the fact that a person is called to develop his physical and spiritual powers in entire manner. Private life includes the right to dispose one's body freely, which is an object to which public authorities are not entitled to dispose in their discretion (ECHR decision in the case of "Pannullo and Forte vs. France" (1997)). On this basis, the ECHR considers that interference with the right to respect for private life is instigated by a medical or psychiatric examination [21]. That is why, in our opinion, along with the examples given above, proceeding examination of a suspect, witness or victim to gather evidence of a crime should also be considered as interfering with one's private life.

Muster is also can be understood as interference into privacy on the grounds that, where it is necessary, it has to be carried out with the assistance of a forensic expert or doctor. This assertion is based on the fact that the ECHR considers any medical intervention to be a private interference, even if it is minor.

In order to reveal the fact that the interference of public authorities into privacy is justified according to the algorithm of D. Vitkauskas, it is necessary to determine whether the interference was quite lawful. To find it out, it is necessary to answer some questions:

1. Is interference of country's internal legal system allowed?

The provisions of parts 1, 2, Article #241 of the CPC of Ukraine require an muster under the following mandatory conditions:

1.1. The muster process is carried out by a certain circle of authorized officials: investigator, prosecutor;

1.2. The muster process is carried out in relation to a certain circle of participants of the criminal proceedings: suspect, witness or victim;

1.3. The legal basis for the muster process is carried out by the prosecutor's decision. Unlike the previous CPC of Ukraine of 1960, this decision can be declared in person or by the request of the investigator (inspector). This creates additional guarantees for the protection of the individual along with ensuring his right to immunity. The order (disposition), containing general information, sets out the circumstances that led to the conduct the muster examination, specifies who is to be mustered and for what purpose;

1.4. If necessary, the muster is carried out with the participation of forensic expert or doctor. The forensic expert or doctor is allowed to perform specific medical knowledge in order to identify, fixate and remove the traces while performing his/her examination tasks for mustering. When involved into conducting muster process, they being specialists, should influence the detection and fixation of traces of a criminal offense and special signs, in particular: to focus the investigator's attention on those features of the body that he did not notice (for example, the presence of a scar or pigmented area of the skin that could be the result destruction or removal of a specific feature); to give characteristics of bodily injury (bruise, wound); to help describe properly the detected damage to the body by accurate indicating their location in accordance with the anatomical structure of the human (for example, the injury is in location of the deltoid muscle of the shoulder); to help determine the shape of the damage, its size, properties, color; to perform actions directly aimed at the removal of detected traces of crime (blood, semen); to consult the investigator (inspector) on the circumstances under which he observes from the point of view of medical knowledge; to express his assumption on the time, the mechanism of occurrence of the detected traces, as well as the possible placement of the victim and the perpetrator at the moment of the formation on the injured body of an examined person; to give explanation of the essence of his actions aimed at identifying, fixing and removing traces of crime or identifying and fixing specific signs; to express considerations about the traces and injuries associated with the commission of a crime, which may be found in other parts of the body or at the crime scene [14].

The peculiarity of the doctor's status as a specialist is also that he must determine the bodily injuries and the nature of the actions that may have endangered the health of the mustered person, which are prohibited by law. At the same time, the doctor may request that his statement of objection be recorded into the protocol concerning the conduct of those activities which, from the point of view of medicine, can be dangerous to the health of that person.

2. Is the relevant legislative provision accessible to the citizen, or has the citizen generally been able to obtain sufficiently fair understanding of the rules of law applicable to the particular case and whether the law is sufficiently precise to allow the citizen to foresee (within reasonable limits) possible consequences of his or her actions [9]?

Having defined the requirements outlined by such criteria as the accessibility of the law to a citizen, it is necessary to point out such national legislative requirements, which it provides:

2.1. Part 3 of Article #241 of the CPC of Ukraine requires that before the muster process begins resolution of the prosecutor on the person to be examined has to be submitted;

2.2. Part 3 of Article #42 of the Criminal Procedure Code of Ukraine provides for the right of the suspect to be clearly and timely informed of his rights, as well as to obtain their clarification. The victim's right to be informed of his/her rights and obligations is enshrined in the Article #56 of the CPC of Ukraine. Rights of witness regulated in Article #66 of the CPC of Ukraine do not provide such rights. Part 3 of Article #223 of the CPC of Ukraine provides for the duties of the investigator, the prosecu-

tor prior to conducting investigative (searching) action to the persons involved in it, explaining their rights and obligations stipulated by the CPC of Ukraine, as well as the responsibilities for those actions established by law. Taking into consideration the above-mentioned, in our opinion, it is to be appropriate, by analogy with the legal regulation of the questioning, in the Article #241 of the CPC of Ukraine, to provide for the duty of the investigator or the prosecutor before mustering of the suspect, the witness or the victim to explain their rights, as well as the purpose and procedure of conducting the examination.

3. Responding to the question whether the interference pursued a legitimate aim is to establish by an authorized officer a factual basis for conducting an examination, namely to conduct a muster to detect traces of crime or special signs on the body. In this case, such a kind of interference serves to protect public order investigation of crime. However, the expediency of achieving this goal through the muster process is given to the investigator or the prosecutor by the legislation.

4. At the same time, while analyzing the provisions of the Article # 241 of the CPC of Ukraine, authors see the difficulty of determining whether the conduct of the muster survey is appropriate to the following criterion, determined in accordance with the algorithm given by D. Vitkauskas, as a measure of convenience. Thus, when analyzing any interference, it is necessary to determine whether it was necessary in a democratic society (in particular, whether it was an adequate response to a public need that is not delayed). In our case, it is understood that a person is offered voluntary examination after the filing of a prosecutor's decision, and in case of refusal, the examination is compulsory. The admissibility of coercion, first of all in the form of physical action, its limits and grounds to participants in the process, who refuse to carry out these investigative (searching) actions is one of the most pressing problems.

When determining whether the objected measures were "necessary in a democratic society", the ECHR noted that the concept of "necessity" for the purposes of the Article #8 of the Convention means, that the interference must meet an urgent social needs and, in particular, remain proportionate to the legitimate goal achieved (ECHR court case decision in "Z vs. Finland" (1997)). The ECHR confirmed that it would consider whether the reasons were justified, appropriate and sufficient in the light of the case in the whole, and whether these measures were proportionate to the legitimate goals. While deciding whether the interference was "necessary", the ECHR would consider the limits of discretion given to public authorities, but it is to be the responsibility of the respondent State to demonstrate the existence of a pressing social position on interference (ECHR court case decision for "Piechowicz vs. Poland" (2012)) [8].

And again, like in the case of determination of expediency, we are facing the fact that the legislator gives the investigator or prosecutor the discretion to determine the appropriateness of compulsory muster. In our opinion, this increases the subjectivism on making legally appropriate decision by an authorized official and engenders grounds for the necessity to its justification proof in case of decision appeal.

With regard to the mentioned-above, it should be noted that in the case of conducting muster process there is no doubt for the need of the final obligatory examination of the suspect, if his body can detect traces of criminal offense or special signs, and if this does not require special forensic examination. To deprive the investigator, the prosecutor and the court from the opportunity to detect these traces while protecting the person's sense of shame means to make it impossible to examine the body of the suspect.

The question of the use of coercion in the course of muster as for witnesses and victims, its admissibility from the point of view of ensuring the human right to non-interfere within the privacy and protection of other person's values is one of the most debated issues in the theory of criminal procedural law. Considerable attention in criminal procedural science has received the opinion of M. S. Strogovich that victims and witnesses, against their will, cannot be forcibly inspected, since the law cares not only for the establishment of truth, but also for it to be attained by means that do not limit the legitimate interests of the individual [12].

According to I. A. Antonov's opinion, the muster process related to the forced exposure of victim's or witnesses' body, which are usually hidden under clothing (especially in cases of sexual offenses) is impermissible in all cases. The following arguments are given against the compulsory examination of witnesses and victims. The violent practicing of it into action is a violation of bodily integrity, part of the integrity of the person guaranteed by the Constitution of Ukraine. Examination process related to exposing a person's body «causes harm to the sense of shame» that must be respected, especially since the victim has already suffered the crime. As a clear example of impermissibility of physical coercion, the author of this point of view states, in particular, that it is forbidden to allow that a woman, rejecting the examination of her body and exerting physical resistance against actions of investigator or doctor, to be forcibly exposed anyway, brought to a gynecological chair and subjected to a compulsory examination or observation [1]. In addition, it can be stated that the use of coercion in such situations is not just unacceptable, but also virtually impossible due to the fact that the actual action here is carried out not by the investigator, but by the doctor who has to overcome the resistance of the examined individual. The doctor is likely to find it impossible to fulfill those actions using force, than to implement the investigator's instructions. Medical ethics, which makes it compulsory for the physician to obtain permission from the patient for the operation, does not allow such a kind of medical manipulations without voluntary consent.

On the contrary to this position I. M. Yanchenko points out that the procedural peculiarities of muster conducting should be the same regardless of the person's procedural status during investigative (searching) actions. This scholar continues that in the case of procedural necessity and lack of other possibilities to establish the facts that are essential to the case, it is considered permissible to carry out a compulsory examination of both the accused (suspect) and the victim (witness) [16]. Denying the use of compulsory muster to the victim, and in some cases to the witness, according to S. S. Klochuriak, significantly violates the suspect's right to defense. An examination of the victim, even in the way of his/her refusal to participate in the conduct of it, is necessary during investigation on rape, robbery, injury and other crimes, especially in cases where the investigator has factual data that can testify to a simulation, a staging of criminal scene offense or reprimand from the side of the victim [4].

In our opinion, the compulsory examination of victims and witnesses can take place only when the refusal of the examination renders the legal assessment of the act impossible and jeopardizes the investigation of the crime and the establishment of circumstances relevant to the criminal proceedings.

Summarizing the above, we consider as necessary to amend part 1 of the Article #241 of the CPC of Ukraine and set it out in the following wording:

«For detection of special features, traces of a criminal offense relevant to criminal proceedings on the body of a person, if this does not require forensic examination, the investigator, the prosecutor shall examine the suspect, as well as the victim and witness with their consent, except muster cases when the examination is necessary to assess their accuracy of proceeding.»

Accordingly, part 3 of the Article #241 of the CPC of Ukraine is expedient to convey in the following wording: "Before commencing the muster, a person to be examined shall be presented with the decree of the prosecutor to explain individual's rights, as well as to ground the purpose and procedure of conducting the examination. Thereafter, the person is offered to undergo a voluntary muster process, and in the case of his/her refusal, the examination is to be processed only in cases provided by the first paragraph of the mentioned Article. Compulsory involvement of a suspect, victim or witness for mustering with the assistance of a forensic expert or a doctor is carried out with the approval of the investigating judge, court.»

Having taken our proposals into account is to serve as additional guarantee against violation of the constitutional rights and freedoms of individuals in criminal proceedings, including the right to non-interference into privacy. Mentioned proposals for amendments to the CPC of Ukraine are to realistically and effectively guarantee adherence to the international legal and constitutional basis for non-interference into privacy during pretrial investigation in a proper way.

Conclusions. Thus, private life is a special sphere of relations that is not subject to external control and is placed outside the law. However, the law is intended to guarantee non-interference of privacy. This provision is particularly relevant in criminal proceedings when there is an excessive risk of violations of the rights and legitimate interests of its participants. Restriction of these rights is possible only in the manner prescribed by the law and solely to achieve the objectives of criminal proceedings in order to protect the state, society and individual citizens from criminal offenses.

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SUMMARY

PROVISION OF THE RIGHT TO NON-INTERFERENCE WITH PRIVACY DURING MUSTER PROCESS WITH THE PARTICIPATION OF DOCTOR (FORENSIC EXPERT)

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The article focuses on the fact that the right to non-interference in privacy is one of the guarantees for the realization of the right to health protection. It is emphasized that the pandemic caused by the SARS-CoV-2 coronavirus has actualized in the democratic countries of the world the question of the permissible limits of state intervention in it, as well as the issue of ensuring during muster process involving a doctor. The purpose of the article is to investigate the concept of the right to non-interference in privacy and the problems of ensuring it, development of proposals to improve the legislation of Ukraine. The methodological ground of the study is based on general and specific methods of scientific knowledge (formal-logical method, comparative-legal, structural-logical). The empirical base of the study is international documents, decisions of the ECHR in medical and legal cases, current legal acts of Ukraine, governing this sphere of legal relations, and assessment of Ukrainian and foreign experts. As a result of the study it was argued that the restriction of the right to non-interference in privacy and subjective legal rights, which are its structural elements, is possible only in such a way that is guaranteed by law, and solely to achieve the goal of protecting the state, society and individual citizens. Based on the analysis of the practice of the ECHR in medical and legal cases, it was found that namely the involvement of a forensic expert or doctor helps to identify and record traces of a criminal offence, special signs, and that these persons can indicate to authorized officials, conducting muster process, the nature of the actions that pose a threat to the health of the person, who is to be mustered and which are prohibited by law, thereby determining the limits of admissibility of intervention. Proposals for amendments and supplements to the Criminal Procedure Code of Ukraine. The conclusions of the article acknowledges that the issues of the right to non-interference in private life, guarantees of the realization of the right to protection of health, and ensuring the right to non-interference in private life during muster process with the participation of doctor should continue a comprehensive investigation to ensure their effective implementation.

Keywords: doctor, assessment, private life, non-interference, forensic expert, protection, guarantees, human rights.

РЕЗЮМЕ

ПРАВО НА НЕВМЕШАТЕЛЬСТВО В ЧАСТНУЮ ЖИЗНЬ КАК ГАРАНТИЯ РЕАЛИЗАЦИИ ПРАВА НА ЗДОРОВЬЕ И ОБЕСПЕЧЕНИЕ ЕГО СОБЛЮДЕНИЯ ПРИ ОСВИДЕТЕЛЬСТВОВАНИИ С УЧАСТИЕМ ВРАЧА (СУДЕБНОГО ЭКСПЕРТА)

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Цель исследования – определение понятия права на невмешательство в частную жизнь и путей его обеспечения, разработка предложений по усовершенствованию законодательства Украины. Методологической основой проведенного исследования явились общие и специальные методы научного познания (формально-логический, сравнительно-правовой, структурно-логический). Эмпирической базой исследования явились международные документы, решения Европейского суда по правам человека в медико-правовых делах, действующие правовые акты Украины, регулирующие эту сферу правоотношений, оценка украинских и зарубежных экспертов. В результате проведенного исследования аргументировано, что ограничение права на невмешательство в частную жизнь и субъективных юридических прав, которые являются его структурными элементами, возможно в порядке, предусмотренном законодательством для защиты государства, общества и отдельных граждан. На основе анализа практики Европейского суда по правам человека в медико-правовых делах установлено, что привлечение судебного эксперта или врача способствует выявлению и фиксации следов уголовного правонарушения. Судебный эксперт или врач могут указать уполномоченным служебным лицам, проводящим освидетельствование, на характер действий, предоставляющих угрозу здоровью человека, в отношении которого проводится освидетельствование, и проведение которых запрещено законом. Авторы рекомендуют внесение изменений и дополнений в Уголовный процессуальный кодекс Украины. Отмечается, что вопросы права на невмешательство в частную жизнь, гарантия реализации права на охрану здоровья, обеспечение права на невмешательство в частную жизнь при проведении освидетельствования с участием врача должны комплексно исследоваться с целью эффективного обеспечения этих прав.

რეზიუმე

პირად ცხოვრებაში ჩაურევლობის უფლება, როგორც ჯანმრთელობის უფლების რეალიზების გარანტია და მისი დაცვის უზრუნველყოფა დამოწმების ჩატარების დროს ექიმის (სასამართლო ექსპერტის) მონაწილეობით

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კვლევის მიზანს წარმოადგენდა პირად ცხოვრებაში ჩაურევლობის უფლების ცნების და მისი უზრუნველყოფის გზების განსაზღვრა, წინადადებების შემუშავება უკრაინის შესაბამისი კანონმდებლობის სრულყოფისათვის. ჩატარებული კვლევის მეთოდოლოგიური საფუძველი არის სამეცნიერო შემეცნების ზოგადი და სპეციალური მეთოდები (ფორმალურ-ლოგიკური, შედარებით-სამართლებრივი, სტრუქტურულ-ლოგიკური). კვლევის ემპირიულ ბაზას შეადგენდნენ საერთაშორისო დოკუმენტები, ადამიანის უფლებათა ევროპული სასამართლოს გადაწყვეტილებები სამედიცინო-სამართლებრივ საქმეებში, უკრაინაში ამ სფეროში სამართლებრივ ურთიერთობათა მარეგულირებელი მოქმედი სამართლებრივი აქტები, უკრაინელი და უცხოელი ექსპერტების შეფასებები. ჩატარებული კვლევის შედეგებით დასაბუთებულია, რომ პირად ცხოვრებაში ჩაურევლობის უფლების და სუბიექტური იურიდიული უფლებების შეზღუდვა, რომლებიც მის სტრუქტურულ ელემენტებს წარმოადგენს, შესაძლებელია კანონით გათვალისწინებულ შემთხვევებში და სახელმწიფოს, საზოგადოებისა და ცალკეული მოქალაქის დაცვის მიზნით. ადამიანის უფლებათა ევროპული სასამართლოს პრაქტიკის ანალიზის საფუძველზე სამედიცინო-სამართლებრივ საქმეებში დადგენილია, რომ სამედიცინო ექსპერტის, ან ექიმის ჩართვა ხელს უწყობს სისხლის სამართლებრივი დანაშაულის კვალის გამოვლენას და დაფიქსირებას. სამედიცინო ექსპერტმა, ან ექიმმა შესაძლოა მიუთითოს დამოწმების ჩამტარებელ უფლებამოსილ სამსახურებრივ პირებს იმ ადამიანის ჯანმრთელობისათვის საფრთხის შემცველი მოქმედებების ხასიათის შესახებ, ვის მიმართაც ტარდება დამოწმება და რომელთა ჩატარებაც აკრძალულია კანონით.

ავტორები რეკომენდებულად თვლიან ცვლილებებისა და დამატებების შეტანას უკრაინის სისხლის სამართლის პროცესუალურ კოდექსში. აღნიშნულია, რომ პირად ცხოვრებაში ჩაურევლობის უფლების, ჯანმრთელობის უფლების რეალიზების გარანტიის და პირად ცხოვრებაში ჩაურევლობის უფლების უზრუნველყოფის საკითხები დამოწმების ჩატარების დროს ექიმის მონაწილეობით გამოკვლეულ უნდა იქნას კომპლექსურად.

КОНТРОЛЬ И НАДЗОР КАК СРЕДСТВА ПРЕДУПРЕЖДЕНИЯ И ВЫЯВЛЕНИЯ ПРАВОНАРУШЕНИЙ В СФЕРЕ ЗДРАВООХРАНЕНИЯ

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С помощью контрольной и надзорной деятельности воплощаются значимые функции публичного управления, благодаря которым государство, через специально созданные органы или путем делегирования соответствующих полномочий органам государственной власти, местного самоуправления или гражданам, оценивает объекты контроля, следит за процессами и процедурами, происходящими в общественно значимых сферах, обеспечивая соблюдение прав, свобод и интересов граждан, способствуя их реализации. К таким значимым сферам общественной жизни, безусловно, относится и сфера здравоохранения, поскольку она обеспечивает право на сохранность здоровья и жизни.

Исследование сущности контрольной и надзорной деятельности в сфере здравоохранения играет значимую роль, так как предназначение такой деятельности заключается в недопущении любых нарушений и отклонений в сфере здравоохранения, непосредственно связанной с жизнедеятельностью населения. Как известно, предоставление неквалифицированных медицинских услуг или же любые отклонения от регламентов в этой деятельности негативно влияют на здоровье человека, обратившегося за медицинской помощью. Это касается как производства медицинских препаратов, так и осуществления иной деятельности в сфере здравоохранения.

По своей сути, содержанию, задачам, субъектами, целями, а также последствиями контроль и надзор являются разными видами деятельности, оба направления следует рассматривать раздельно, с учетом возможности их соотношения и того факта, что надзорная деятельность может являться частью контролирующей функции определенного органа. Учитывая значимость контрольной и надзорной деятельности в сфере здравоохранения, примечательно, что основные положения, регламентирующие осуществление этих видов деятельности, определены в Законе Украины

«Основы законодательства Украины о здравоохранении» (далее – Закон № 2801-XII) «государство через специально уполномоченные органы исполнительной власти осуществляет контроль и надзор за соблюдением законодательства о здравоохранении, государственных стандартов, критериев и требований, направленных на обеспечение здоровой окружающей природной среды и санитарно-эпидемиологического благополучия населения, нормативов профессиональной деятельности в сфере здравоохранения, требований Государственной Фармакопеи, стандартов медицинского обслуживания, медицинских материалов и технологий» [8].

Целью исследования явилось раскрыть сущность и особенности контрольной и надзорной деятельности как средства предупреждения и выявления правонарушений в сфере здравоохранения. Для достижения цели поставлены задачи: анализ понятий «контроль и надзор» как способов обеспечения законности в сфере здравоохранения, выявить их отличительные признаки, охарактеризовать субъекты осуществления контрольно-надзорной деятельности, проанализировать объекты государственного контроля и общественного надзора в сфере здравоохранения.

Материал и методы. Материалом исследования являлись политико-правовая публицистика, труды отечественных ученых, аналитические материалы, справочные издания, Интернет-ресурсы и украинское законодательство, регулирующее контрольно-надзорную деятельность в сфере здравоохранения.

Методологическую основу статьи составили общенаучные и специальные методы научного познания, применение которых обусловлено целью, задачами, спецификой предмета и объекта исследования: диалектический метод, использование которого наряду со структурно-логическим методом и методом моделирования позволяет определить современное состояние контрольно-надзорной деятельности в рассматриваемой нами сфере, выявить её основные проблемы и определить перспективы их научно-практического разрешения. Метод структурно-функционального анализа позволяет разграничить контроль и надзор как способы обеспечения законности в сфере здравоохранения по ряду признаков, в частности субъектам и результатам осуществления обоих видов деятельности. Благодаря системно-структурному методу осуществляется классификация субъектов и объектов контроля (надзора) в сфере здравоохранения.

Результаты и обсуждение. Прежде чем перейти непосредственно к контролю и надзору в сфере здравоохранения следует отметить, что под контролем понимается сложный процесс, который состоит из совокупности различных действий и мер, характерных и для надзора. Надзор предполагает более простые действия в виде наблюдения, сбора и оформления информации, при этом не предусмотрено какого-либо непосредственного влияния на его объект, тогда как при контроле такое влияние возможно. В процессе контрольной деятельности в случае необходимости могут осуществляться как надзор, так и отдельные характерные для него действия, в частности мониторинг или консолидация данных, касающихся объекта контроля.

Контроль является одним из действенных и эффективных средств обеспечения законности, так как с его помощью осуществляется не только наблюдение за соблюдением установленных норм, стандартов и требований подконтрольными объектами, но и анализ и поиск проблем в их деятельности, прогнозирование, оптимизация и корректировка вопросов, возникающих в их деятельности. Минздраву как субъекту контрольных полномочий следует концентрировать усилия на соблюдении клинических протоколов и стандартов подчиненными ему учреждениями, своевременно корректируя их деятельность.

Надзор как самостоятельная деятельность является более узким, поскольку сводится к пассивной форме наблюдения. Определяющим критерием разграничения понятий «контроль» и «надзор» является возможность воздействия на объект: при контроле субъект может оказывать непосредственное влияние на состояние или его деятельность, а во время надзора такое влияние невозможно – информация об имеющихся отклонениях и нарушениях передается специально уполномоченному органу.

Субъекты правоотношений в сфере здравоохранения в виде государства и его уполномоченных органов в зависимости от направлений государственной политики подразделяются на субъектов, которые формируют, реализуют и осуществляют контроль и надзор в этой сфере.

В Законе № 2801-XII предусмотрено, что государство через специально уполномоченные органы исполнительной власти осуществляет контроль и надзор за соблюдением законодательства о здравоохранении [8]. Так, органом контроля и надзора в сфере здравоохранения является Министерство Здравоохранения Украины (далее – Минздрав), которое согласно положению: утверждает отраслевые стандарты в области здравоохранения (клинические протоколы и стандарты) и контролирует их выполнение; осуществляет контроль за соблюдением единых требований и критериев в учреждениях здравоохранения системы экстренной медицинской помощи, за деятельностью психиатрических учреждений независимо от формы собственности, за соблюдением законодательства о здравоохранении [10].

Минздрав, в пределах своих полномочий, осуществляет контрольно-надзорные мероприятия с целью оперативного выявления проблем и нарушений действующего законодательства в сфере здравоохранения, способствует их решению и заблаговременному устранению до момента причинения возможного вреда лицам, их правам и свободам. Указанные процедуры осуществляются в сфере производства, контроля качества и реализации лекарственных средств, в частности в лечебно-профилактических учреждениях, аптеках медицинских иммунобиологических препаратов и медицинских изделий, наркотических средств, психотропных веществ, их аналогов и прекурсоров. Контролирует соблюдение законодательства в сфере здравоохранения, включая клинические протоколы и стандарты, выполнение требований к учреждениям системы здравоохранения, оказывающих экстренную медицинскую помощь; физическое воспитание учащихся в общеобразовательных учебных заведениях (такой контроль носит медико-педагогический характер); деятельность психиатрических учреждений и работников, оказывающих психиатрическую помощь; деятельность учреждений здравоохранения и научных учреждений, осуществляющих деятельность, связанную с трансплантацией органов, тканей и клеток; качество крови и ее компонентов; целевого использования этилового спирта, используемого при производстве лекарственных средств для медицинской практики; использование финансовых и материальных ресурсов; сохранение государственной тайны и информации с ограниченным доступом.

Минздрав осуществляет также инспекционные процедуры, содержание которых относится к контрольно-надзорной деятельности. Такие процедуры необходимы для

предотвращения совершения правонарушений субъектами, которые подлежат проверке на основании обращения лиц, считающих, что такие нарушения имели место, или при самостоятельном выявлении нарушений норм национального законодательства. Например, Гослекслужба систематически проводит проверку процесса и условий производства лекарственных средств. В контексте этой процедуры, под инспектированием понимается «процедура оценки соответствия фармацевтической системы качества субъекта хозяйствования и фактического состояния имеющихся условий производства лекарственных средств и условий контроля качества действующим в Украине требованиям надлежащей производственной практике по месту осуществления деятельности (местонахождению производственных мощностей, в том числе зон контроля качества и зон хранения по контракту (договору)» [17]. При проведении инспектирования важно установить соответствие той или иной деятельности требованиям надлежащей производственной практики - Good Manufacturing Practice (GMP), которая является «частью обеспечения качества, гарантирующего, что продукцию постоянно производят и контролируют по стандартам качества, соответствующим ее назначению и требованиям регистрационного досье или спецификации на эту продукцию» [7].

Следовательно, в сфере здравоохранения осуществляются контрольно-надзорные процедуры, в основном, связанные с медицинским и медико-педагогическим контролем, государственным санитарно-эпидемиологическим надзором и административным контролем, который имеет место при организации работы в аппарате Минздрава и медицинских учреждениях.

Оценка деятельности самого Минздрава осуществляется Комитетом Верховной Рады Украины по вопросам здравоохранения, так, в материалах заседания Комитета от 05.02.2019 г. указано: «заслушав вопрос об эпидемической ситуации с заболеваемостью населения корью, Комитет принял решение: признать противоэпидемические меры, принимаемые Минздравом Украины с целью ликвидации вспышки инфекции кори, неудовлетворительными и содержащими опасность для жизни и здоровья украинских граждан, а также безопасности государства; признать работу оперативного штаба Минздрава Украины по реагированию на ситуацию по распространению кори в Украине неудовлетворительной» [13]. Этот пример указывает на существование правоотношений контроля между Комитетом Верховной Рады Украины по вопросам здравоохранения и Минздрава. Публично объявленная информация о деятельности Минздрава предоставляет возможность обществу определиться относительно оценок государственного управления в сфере здравоохранения. Такой подход позволяет рассматривать правоотношения контроля в сфере здравоохранения как сложные, в которых несколько субъектов имеют полномочия по оценке деятельности Минздрава.

Контроль и надзор в сфере здравоохранения является самостоятельным направлением деятельности в государстве, поэтому целесообразно выделять и специальные органы, которые могут осуществлять такую деятельность. Так, обязывающим субъектом относительно Минздрава является Национальная служба здоровья Украины (далее – НСЗУ) – государственный орган, полномочия которого определены в постановлении Кабмина «Об образовании Национальной службы здоровья Украины» от 27.12.2017 № 1101 [21]. НСЗУ наделена контролирующими полномочиями (борьба с коррупцией, организация внутреннего контроля и внутреннего аудита, контроль за использованием финансовых и материальных ресурсов НСЗУ т.д.). Однако контрольные полномочия НСЗУ ограничены реализацией государственной политики по финансированию медицинского обслуживания населения.

Государственная служба Украины по лекарственным средствам и контролю за наркотиками (далее – Гослекслужба) согласно положению о нем осуществляет государственный контроль за соблюдением требований законодательства по обеспечению качества и безопасности лекарственных средств и медицинских изделий на всех этапах обращения, в том числе правил осуществления надлежащих практик (производственная, дистрибьюторская, хранение, аптечная) за ввозом на таможенную территорию Украины лекарственных средств; соблюдением субъектами хозяйствования лицензионных условий осуществления хозяйственной деятельности по производству лекарственных средств, их импорта оптовой и розничной торговли лекарственными средствами, другие виды контроля [9].

Цель существования этой службы совсем иная, чем НСЗУ. Если НСЗУ реализует государственную политику по финансированию медицинского обслуживания населения, то основными полномочиями Гослекслужбы являются контролирующие. При этом эти государственные органы имеют тесную взаимосвязь в своей деятельности, поскольку Гослекслужба обеспечивает контроль за качеством медицинского оборудования и лекарственных средств, используемых для лечения пациентов, а следовательно, является элементом в политике финансирования здравоохранения.

Анализируя полномочия Гослекслужбы, следует сделать вывод, что это прежде всего орган государственного контроля (надзора). В Законе Украины «Об основных принципах государственного надзора (контроля) в сфере хозяйственной деятельности» закреплено, что государственный надзор (контроль) – это «деятельность уполномоченных законом центральных органов исполнительной власти ... в пределах полномочий, предусмотренных законом, по выявлению и предотвращению нарушений требований законодательства субъектами хозяйствования и обеспечения интересов общества, в частности надлежащего качества продукции, работ и услуг, допустимого уровня опасности для населения, окружающей природной среды» [19].

Из этого следует, что Гослекслужба осуществляет свою деятельность по выявлению и предотвращению незаконной деятельности в сфере обращения лекарственных и наркотических препаратов. Возможность осуществления контроля непосредственно связана с полномочиями лицензирования соответствующей хозяйственной деятельности в сфере фармации и производства медицинского оборудования. Согласно Закону Украины «О лицензировании видов хозяйственной деятельности», лицензирование – это средство государственного регулирования производства видов хозяйственной деятельности, подлежащих лицензированию, направленное на обеспечение реализации единой государственной политики в сфере лицензирования, защиту экономических и социальных интересов государства, общества и отдельных потребителей [18]. Исходя из этого, Гослекслужба является органом государственного контроля (надзора) и органом лицензирования.

Подытоживая вышеизложенное, следует подчеркнуть, что Гослекслужба является специальным органом исполнительной власти в форме юридического лица публичного

права, который осуществляет контроль (надзор), лицензирование хозяйственной деятельности в сфере фармацевтических, наркотических препаратов и изделий медицинского назначения, противодействия их незаконному обороту, формирует и ведет государственные реестры.

Считаем целесообразным внести изменения в Положение о Гослекслужбе путем совершенствования ее контрольных функций. Предлагаем Гослекслужбу наделить правом проверять деятельность учреждений здравоохранения и других субъектов хозяйствования, осуществляющих взятие, переработку, хранение, реализацию и применение донорской крови и препаратов, а также выяснять, соблюдены ли правила контроля за безопасностью и качеством донорской крови, ее компонентов, препаратов и соответствующих консервирующих растворов, порядка обмена донорской кровью, ее компонентами и препаратами и вывоза их за пределы Украины, порядка медицинского обследования донора перед сдачей крови и ее компонентов. Следует усовершенствовать положения Порядка контроля за соблюдением показателей безопасности и качества донорской крови и ее компонентов [16], который перегружен техническими нормами, привести его в соответствие со ст. 45-1 КУоАП и четко выписать контрольные функции субъектов контроля и алгоритм его осуществления.

Таким образом, государство при осуществлении контроля и надзора в сфере здравоохранения реализует эти возможности через общий орган управления – Минздрав и специально созданные для этого органы – Гослекслужба, НСЗУ.

Наряду с рассмотренными нами субъектами, для которых обеспечение функционирования здравоохранения является основным направлением их деятельности, существуют государственные органы для которых этот вид деятельности не является основным. Речь идет об органах местного самоуправления, объединенных территориальных общинах и негосударственных институтах (например, Ассоциация городов Украины, Ассоциация французско-украинского сотрудничества в сфере здравоохранения и фармации, общественная организация «Украинская Ассоциация общественного здоровья»).

Так, согласно Закону Украины «Об основных принципах государственного надзора (контроля) в сфере хозяйственной деятельности» органы местного самоуправления отнесены к органам государственного контроля [19]. Будучи наделены властными полномочиями, они осуществляют контролирующую функцию в сфере здравоохранения. Осуществление такого контроля, как одного из средств управления, позволяет выявить факты коррупции, а также нарушения прав и законных интересов граждан в сфере здравоохранения, состояние заболеваемости на определенной территории, что является залогом своевременного принятия необходимых мер для предотвращения негативных последствий.

Проанализировав Положение об Отделе здравоохранения Сумского городского совета, можно выявить наличие контролирующей функции этого органа местного самоуправления, в частности: за состоянием здоровья детей в детских дошкольных и школьных учебных заведениях независимо от их ведомственного подчинения; за состоянием здоровья лиц, занимающихся физической культурой и спортом; за выполнением работ по строительству и капитальному ремонту учреждений здравоохранения, учреждений и предприятий; за рациональным использованием бюджетных средств в этих учреждениях согласно утвержденному бюджету на текущий год [11]. Из приведенного примера можно сделать вывод, что контроль органов местного самоуправления в сфере здравоохранения существует как самостоятельная функция.

Объектами государственного контроля в сфере здравоохранения являются:

1. *Законодательство,* т.е. совокупность всех нормативно-правовых актов, содержащих нормы, направленные на урегулирование общественных отношений в рамках системы здравоохранения. Каждая норма закона и подзаконного нормативно-правового акта, связанная с обеспечением прав граждан на жизнь, здоровье, их охрану, медицинскую помощь и страхование, гарантированных Конституцией Украины [6].

2. *Окружающая среда.* Так, согласно Водного кодекса Украины Центральный орган исполнительной власти, реализующий государственную политику в сфере санитарного и эпидемического благополучия населения, во время возникновение аварийных ситуаций согласовывает решение «о сбросе сточных вод с накопителей в водные объекты, если это не приведет к превышению нормативов экологической безопасности водопользования» [1]. Кроме того, указанный орган осуществляет систематический контроль за качественными показателями воды, предназначенной для нецентрализованного водоснабжения населения, согласовывает разрешение на срастание сельскохозяйственных угодий и создание полигонов для отходов производства [1]. Контроль водопользования частично реализуется Минздравом путем нормализации деятельности по водопользованию посредством разработки и утверждения нормативов экологической безопасности [1].

3. *Санитарно-эпидемическое благополучие населения,* в частности: а) состояние здоровья населения, под которым необходимо понимать показатели оценки и условия развития его физических, психических и социальных функций; б) среда жизнедеятельности человека - те природные или искусственные факторы и условия, которые окружают человека в процессе повседневной деятельности; в) определенные показатели, характеризующие факторы среды жизнедеятельности, которые могут прямо или косвенно повлиять на здоровье человека [5]. Основным субъектом, осуществляющими контрольную деятельность за санитарно-эпидемиологическим благополучием населения, является Минздрав, который согласно Положению о нём [15], утверждает государственные, санитарные, противоэпидемические, гигиенические нормы, правила, нормативы и регламенты; проводит расследование причин и условий возникновения инфекционных заболеваний, отравлений, радиационных аварий; обеспечивает проведение государственного социально-гигиенического мониторинга.

Широкие полномочия по осуществлению контрольной и надзорной деятельности имеет Государственная служба Украины по вопросам безопасности пищевых продуктов и защиты потребителей (далее – Держпродспоживслужба), которая непосредственно осуществляет так называемый предохранительный и текущий государственный санитарно-эпидемиологический надзор [2]. Речь идет о контрольной деятельности «за соблюдением юридическими и физическими лицами санитарного законодательства с целью предупреждения, выявления, уменьшения или устранения вредного влияния опасных факторов на здоровье людей и по применению мер правового характера к нарушителям» [12]. Следует отметить, что рассматриваемая деятельность хотя и определяется как государственный санитарно-эпидемиологический надзор, однако имеет признаки не надзор-

ной, а контрольной деятельности, поскольку речь идет об «активных действиях» (согласование, проверка, экспертиза) в указанном процессе, а также возможности влиять на объект контроля. Подтверждением осуществления подобной активной деятельности может быть «расследование причин и условий возникновения инфекционных болезней, профессиональных заболеваний, массовых неинфекционных заболеваний (отравлений), радиационных поражений людей; назначение государственной санитарно-эпидемиологической экспертизы, а в случае необходимости определение состава комиссии для ее проведения; внесение обязательных для выполнения предложений по устранению выявленных нарушений санитарного законодательства, осуществлению в установленном законом порядке производства по делам о нарушении санитарного законодательства; издание постановлений о наложении штрафа и, в предусмотренных законом случаях, применение финансовых санкций за нарушение санитарного законодательства [14].

В то же время возможность осуществления решающего влияния на объекты «надзора» предусмотрена в Законе Украины «Об обеспечении санитарного и эпидемического благополучия населения» путем ограничения, временного запрета, прекращения или приостановления их деятельности, если они создают угрозу нарушением установленных санитарных норм, в частности: «ограничение, временный запрет или прекращение строительства, реконструкции и расширения объектов в случае отступления от утвержденного проекта; ограничения, приостановления или запрет выбросов (сбросов) загрязняющих веществ при условии нарушения санитарных норм; приостановление или прекращение инвестиционной деятельности в случаях, установленных законодательством» [12]. Следовательно, государственный санитарно-эпидемический надзор по сути является не надзорной, а контрольной деятельностью. Поэтому определять его как надзор нецелесообразно, поскольку это противоречит его содержательной сущности и является терминологической ошибкой законодательства [5].

4. *Нормативы профессиональной деятельности в сфере здравоохранения.* Следует отметить, что в сфере здравоохранения объект контроля не связан только с врачами, оказывающими медицинскую помощь. Он имеет более широкий ареал и касается трудовой деятельности всех субъектов здравоохранения, связанной с процессами «сохранения и восстановления физиологических и психологических функций, оптимальной трудоспособности и социальной активности человека при максимальной биологически возможной индивидуальной продолжительности его жизни» [8]. В этом случае объект контроля включает деятельность иных работников системы здравоохранения: а) руководителей лечебно-профилактических учреждений, производственных подразделений в бытовом обслуживании, деятельность которых осуществляется в сфере оказания медицинской помощи); б) профессионалов в области лечебного дела и педиатрического профиля, стоматологии, фармации, медико-профилактического дела и других сфер медицины; в) специалистов - акушерок, инструкторов-дезинфекторов, инструкторов по трудовой терапии, лаборантов по иммунологии, лаборантов судебно-медицинской лаборатории, оптометристов, рентгенолаборантов, медицинских сестер, медицинских статистиков, техников-ортезистов-гипсувальников, фармацевтов, фельдшеров; г) технических служащих - медицинских регистраторов, сестер-хозяек; д) рабочих - сестер-хозяек, младших медицинских сестер по уходу за больными [4].

Контроль за нормативами профессиональной деятельности в сфере здравоохранения осуществляется в области теоретических знаний, умений, практических навыков и профессиональной этики, причем их объем является нормативным и закреплен в различных положениях нормативно-правовых актов в сфере здравоохранения. Такой контроль осуществляется в пределах экзаменационных процедур, а также фактически всеми лицами, занимающими руководящие должности относительно их подчиненных.

5. *Процесс производства и качества лекарственных средств.* Основной контроль за указанной деятельностью осуществляет Минздрав путем утверждения: «общих требований к материально-технической базе для производства лекарственных средств, осуществления производственного контроля их качества, а также технологических регламентов; посредством методов осуществления контроля качества лекарственных средств; государственных и отраслевых стандартов качества лекарственных средств, медицинских иммунобиологических препаратов» [15]; проведением инспектирования качества лекарственных средств и медицинских изделий.

Непосредственный контроль, который проводит Гослекслужба, осуществляется в следующих направлениях: 1) нормативном - разработка проектов государственных программ, включая сферу оборота наркотических средств, психотропных веществ и прекурсоров; проверка соблюдения требований действующего законодательства в сфере качества и безопасности лекарственных средств и других медицинских изделий; 2) превентивном - осуществление отраслевой аттестации лабораторий, ответственных за качество лекарственных средств; контроль за импортом лекарственных средств; применение мер, направленных на устранение нарушений в сфере оборота наркотических и других средств; 3) информационном - проведение обмена данными на международном уровне по контрольной деятельности в сфере оборота наркотических веществ.

6. *Процессы, осуществляемые в пределах медицинского обслуживания*. Речь идет о «деятельности учреждений здравоохранения и физических лиц-предпринимателей, зарегистрированных и получивших соответствующую лицензию в установленном законом порядке, в сфере здравоохранения, не обязательно ограничивающейся медицинской помощью, но непосредственно связанной с ее предоставлением» [8]. Контроль и надзор за этим объектом осуществляет Минздрав, который следит «за соблюдением законодательства о здравоохранении, в частности о медико-социальной экспертизе, требованиях, необходимых для осуществления деятельности, связанной с трансплантацией органов и других анатомических материалов человеку, и соблюдением отраслевых стандартов медицинского обслуживания, медицинских материалов и технологий» [15].

7. *Медицинские материалы и технологии.* Это достаточно широкая сфера, включающая биомедицинские (биомедицинские металлы, полимеры, керамика) и интеллектуальные (сплавы, имеющие эффект памяти формы; полимеры; клетки; ткани; искусственные органы; системы обмена; лекарственные препараты; медицинские изделия и устройства; методы диагностики и реабилитации; хирургические процедуры; медицинская пропаганда); медицинские материалы. Поэтому субъекты и формы контроля зависят от каждого конкретного объекта.

В системе управления здравоохранением, в процессе реализации медицинской реформы в Украине, приобретают актуаль-

ность контроль и надзор со стороны общественности и медицинского сообщества. При этом «общественный контроль» и «общественный надзор» ограниченны по своей сути и не позволяют пока считать общественность эффективным субъектом властных полномочий в сфере здравоохранения.

Субъектом, не имеющим властных полномочий, но способствующим реализации права граждан на участие в управлении здравоохранением, является наблюдательный совет учреждения здравоохранения. Согласно Закону № 2801-XII «при государственных и коммунальных учреждениях здравоохранения, оказывающих медицинскую помощь вторичного и третичного уровней, с которыми главными распорядителями бюджетных средств заключены договора о медицинском обслуживании населения, по решению собственника учреждения здравоохранения (уполномоченного им органа) образуются наблюдательные советы с обязательным привлечением представителей общественности естественно с их согласия. Наблюдательные советы также могут образовываться по решению собственника при других учреждениях здравоохранения». Объектами их наблюдения являются: соблюдение прав, свобод и обеспечения безопасности пациентов; соблюдение требований законодательства, регламентирующего предоставление медицинских услуг учреждениями системы здравоохранения; финансовая и хозяйственная деятельность учреждений здравоохранения [8].

В Типовом Положении о наблюдательном совете учреждения здравоохранения, утвержденного постановлением Кабмина от 27.12.2017 № 1077, закреплены полномочия наблюдательного совета: рассмотрение вопросов относительно соблюдения законодательства в сфере медицинского обслуживания, в частности соблюдение прав пациентов, рассмотрение их заявлений (жалоб), финансовой и хозяйственной деятельности соответствующего учреждения системы здравоохранения; предоставление предложений руководителю учреждения системы здравоохранения по совершенствованию его деятельности, наличии недостатков, включая факты нарушения или несоблюдения действующего законодательства и их устранения [20]. Сам по себе наблюдательный совет никак не может влиять на объект своей деятельности. Об этом свидетельствует его специфический «метод воздействия» – через руководителя учреждения здравоохранения, которому он сообщает о результатах своей работы. Следует подчеркнуть, что суть такой деятельности по своему содержанию в полном объеме совпадает с содержанием понятия «надзор». К тому же в указанном типовом положении содержится прямое указание на то, что наблюдательный совет учреждения здравоохранения является надзорным органом.

Для обеспечения прозрачности и общественного контроля за деятельностью НСЗУ образуется Совет общественного контроля при НСЗУ (далее – Совет). Полномочия Совета определены в Положении о Совете, утвержденном постановлением Кабмина от 28.03.2018 № 271 [2], согласно которому основными задачами Совета являются: 1) осуществление общественного контроля за деятельностью НСЗУ; 2) содействие взаимодействию НСЗУ с общественными объединениями, другими институтами гражданского общества в сфере медицинского обслуживания населения; 3) обеспечение прозрачности деятельности НСЗУ; 4) информирование НСЗУ об общественном мнении по формированию и реализации государственной политики в сфере государственных финансовых гарантий медицинского обслуживания населения и о программе государственных гарантий медицинского обслуживания населения. Для реализации этих задач Совет осуществляет взаимодействие с общественностью, средствами массовой информации, международными организациями. Это взаимодействие возможно благодаря публичным задачам, распространению информации о деятельности НСЗУ, проведению общественной экспертизы решений НСЗУ, общественного контроля за средствами государственного бюджета [2]. Анализируя полномочия Совета, очевидно, что они направлены на проведение и реализацию общественного контроля. Повлиять на деятельность государственных органов этот орган общественного контроля может только путем публичного обсуждения проблемных вопросов, выявленных в деятельности НСЗУ. Кроме того, он может назначать проведение общественной экспертизы. По нашему мнению, наличие органов общественного контроля, независимых от государственных органов, является проявлением построения демократического государства, в котором бремя контроля сбалансировано распределяется между государственными и общественными органами. Общественный контроль позволяет избежать государственного влияния на принятие решений, обойти коррупционный элемент. Несмотря на положительные аспекты общественного контроля, следует отметить, что содержание правоотношений, возникающих между НСЗУ и Советом будет иметь негативный характер, поскольку уполномоченное лицо (Совет) не имеет прямых полномочий по прекращению деятельности НСЗУ, привлечению его должностных лиц к ответственности, а обязывающее лицо (НСЗУ) не имеет прямой корреспондирующей юридической обязанности по реагированию на оценку его деятельности.

Вывод. Подытоживая изложенное выше, следует отметить, что основными способами обеспечения законности в сфере здравоохранения являются контроль и надзор, которые различаются по ряду признаков, в частности субъектам и результатам осуществления обоих видов деятельности. Государственный контроль в сфере здравоохранения осуществляют органы государственной власти, прежде всего Минздрав и Гослекслужба. Одной из новаций реформирования системы здравоохранения является создание в 2017 г. нового центрального органа исполнительной власти, реализующего государственную политику в сфере государственных финансовых гарантий медицинского обслуживания населения - НСЗУ. Указано, что контрольные полномочия НСЗУ в основном касаются внутреннего организационного аспекта функционирования собственного аппарата по выполнению задач в сфере гражданской защиты; соблюдения антикоррупционного законодательства; использования финансовых и материальных ресурсов.

Выделены объекты государственного контроля в сфере здравоохранения: 1) медицинское законодательство; 2) окружающая среда; 3) санитарно-эпидемическое благополучие населения; 4) нормативы профессиональной деятельности в сфере здравоохранения; 5) процесс производства и стандарты качества лекарственных средств; 6) процессы, осуществляемые в пределах медицинского обслуживания; 7) медицинские материалы и технологии.

В статье указано, что контроль в сфере здравоохранения вместе с государственными органами осуществляет и общественность. При этом обосновано, что участие общественности в здравоохранении тяготеет к надзорной деятельности. Поэтому понятие «надзор» следует применять исключительно относительно осуществления общественно-

го контроля в сфере здравоохранения. Речь идет об участии общественности в составе специально созданных наблюдательных советов при учреждениях здравоохранения и Совете общественного контроля при НСЗУ.

Объектами общественного надзора являются: а) соблюдение прав, свобод и обеспечения безопасности пациентов; б) соблюдение требований законодательства, регламентирующего предоставление медицинских услуг учреждениями системы здравоохранения; 3) финансовая и хозяйственная деятельность учреждений здравоохранения.

В то же время, контроль и надзор имеют общей целью предупреждение и выявление правонарушений в сфере здравоохранения, в результате чего лица, виновные в их совершении, привлекаются к юридической ответственности. Считаем необходимым пересмотреть объем штрафных санкций за совершение административных правонарушений в сфере здравоохранения в сторону увеличения.

Обосновано, что контрольно-надзорные процедуры в сфере здравоохранения связанны преимущественно с медицинским и медико-педагогическим контролем, административным контролем и государственным санитарно-эпидемиологическим надзором.

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SUMMARY

CONTROL AND SUPERVISION AS MEANS FOR PREVENTING AND DETECTING OFFENSES IN THE HEALTH CARE SECTOR IN UKRAINE

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The purpose of the article is to reveal the essence and specific features of control and supervisory activities as means for preventing and detecting offenses in the health care sector on

the basis of existing theoretical concepts and approaches. The research materials were political and legal opinion journalism, works of national scholars, analytical materials, reference publications, Internet resources and Ukrainian legislation regulating control and supervisory activities in the health care sector. The methodological basis of the article was formed by a set of general scientific (dialectical, etc.) and special (system and structural, structural and logical analysis, synthesis, etc.) methods of scientific cognition.

It has been concluded that the main ways of ensuring the rule of law in the health care sector are control and supervision, which are distinguished in a number of characteristics, in particular, the subjects and the results of implementing both types of activities. State control in the health care sector carried out by state authorities (Ministry of Health, State Medical Service, National Health Service of Ukraine), and public supervision carried out by the representatives of the public in the health care sector (supervisory boards at health care institutions, the Council of Public Control under the National Health Service of Ukraine, etc.) have been characterized. The objects of state control and objects of public supervision in the health care sector in Ukraine have been specified and analyzed.

Keywords: health care sector, control and supervision in the health care sector, subjects of state control in the health care sector, objects of control and supervision in the health care sector, offenses in the health care sector.

РЕЗЮМЕ

КОНТРОЛЬ И НАДЗОР КАК СРЕДСТВА ПРЕДУПРЕЖДЕНИЯ И ВЫЯВЛЕНИЯ ПРАВОНАРУШЕНИЙ В СФЕРЕ ЗДРАВООХРАНЕНИЯ

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Цель исследования - на основании существующих теоретических представлений и подходов раскрыть сущность и особенности контрольной и надзорной деятельности как средства предупреждения и выявления правонарушений в сфере здравоохранения. Материалом исследования явились политико-правовая публицистика, труды украинских ученых, аналитические материалы, справочные издания, интернет-ресурсы и украинское законодательство, регулирующее контрольно-надзорную деятельность в сфере здравоохранения. Методологическую основу статьи составили совокупность общенаучных (диалектический) и специальных (системно-структурный, структурно-логический анализ, синтез) методов научного познания.

В результате проведенного исследования делается вывод, что основными способами предупреждения правонарушений в сфере здравоохранения являются контроль и надзор, которые различаются по ряду признаков, в частности субъектам и результатам осуществления обоих видов деятельности. Охарактеризованы государственный контроль в сфере здравоохранения, осуществляемый органами государственной власти (Минздрав, Гослекслужба, Национальная служба здоровья Украины), и общественный надзор, осуществляемый представителями общественности в сфере здравоохранения (наблюдательные советы при учреждениях здравоохранения, Совет общественного контроля при Национальной службе здоровья Украины). Выделены и проанализированы объекты государственного контроля и общественного надзора в сфере здравоохранения в Украине.

რეზიუმე

კონტროლი და **მეთვალყურეობა,როგორც საშუალებები ჯანმრთელობის დაცვის სფეროში სამართალდარღვევათა** თავიდან აცილებისა და გამოვლენისათვის

[1]ვ.ტერემეცკი, [2]ტ.ნიკოლაენკო, [3]გ.დიდკოვსკაია, [3]ა.გმირინი, [4]ტ.შაპოვალი

[1]დასავლეთ უკრაინის **ეროვნული უნივერსიტეტი, ტერ**ნოპოლი; [2]უკრაინის სახელმწიფო სასაზღვრო სამსახურის ბოგდან ხმელნიცკის სახ. ეროვნული აკადემია; [3]უკრაინის **სახელმწიფო ფისკალური სამსახურის უნი**ვერსიტეტი, ირპენი; [4]ჩერკასკის ბოგდან ხმელნიცკის სახ. ეროვნული უნივერსიტეტი, უკრაინა

კვლევის მიზანს წარმოადგენდა კონტროლის და მეთვალყურეობითი საქმიანობის, როგორც ჯანმრთელობის დაცვის სფეროში სამართალდარღვევათა თავიდან აცილებისა და გამოვლენის საშუალებების, არსისა და თავისებურებების ახსნა არსებული თეორიული წარმოდგენებისა და მიდგომების საფუძველზე. კვლევის მასალას წარმოადგენდა პოლიტიკურ-სამართლებრივი პუბლიცისტიკა, უკრაინელი მეცნიერების შრომები, ანალიტიკური მასალები, საცნობარო გამოცემები, ინტერნეტ-რესურსები და საკონტროლო-სამეთვალყურეო საქმიანობის მარეგულირებელი კანონმდებლობა უკრაინაში. სტატიის მეთოდოლოგიურ საფუძველს წარმოადგენს სამეცნიერო შემეცნების ზოგადი (დიალექტიკური) და სპეციალური (სისტემურ-სტრუქტურული, სტრუქტურულ-ლოგიკური ანალიზი, სინთეზი) მეთოდების ერთობლიობა.

ჩატარებული კვლევის საფუძველზე ავტორები დაასკვნიან, რომ ჯანმრთელობის დაცვის სფეროში სამართალდაღრვევათა თავიდან აცილების ძირითად საშუალებას წარმოადგენს კონტროლი და მეთვალყურეობა, რომელნიც რიგი ნიშნებით განსხვავდება, კერძოდ, საქმიანობის ორივე ფორმის სუბიექტებით და განხორციელების შედეგებით. დახასიათებულია სახელმწიფო კონტროლი ჯანმრთელობის დაცვის სფეროში, განხორციელებული ხელისუფლების ორგანოთა მიერ (ჯანდაცვის სამინისტრო, უკრაინის ჯანმრთელობის ეროვნული სამსახური) და საზოგადოებრივი მეთვალყურეობა, განხორციელებული საზოგადოების წარმომადგენლების მიერ (ჯანდაცვის ორგანიზაციებთან არსებული სამეთვალყურეო საბჭოები, უკრაინის ჯანმრთელობის ეროვნული სამსახურის საზოგადოებრივი კონტროლის საბჭო). გამოყოფილი და გაანალიზებულია სახელმწიფო კონტროლის და საზოგადოებრივი მეთვალყურეობის ობიექტები ჯანმრთელობის დაცვის სფეროში უკრაინაში.