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EDITORIAL

Wound Management with Chitosan Based Biomaterials

Dmitry Domenyuk

Executive Editor,
Archiv EuroMedica

Dear clinicians, researchers, colleagues and friends!

Treatment of wounds poses acute medical and social challenges for research and practice. It acquires even more importance with an increase in natural disasters, industrial and domestic accidents and military conflicts.

In modern medicine there are a great number of wound dressings. Their application is based on the principle of moist recovery of the wound formulated by G. Winter (1962) and C.D. Hinman, H. Maibach (1963). The authors established that moist antibacterial environment was optimal for maintenance of normal mitotic process in the wound: migration of epidermal cells and epithelialization of wounds occur more actively in the moist environment and not in the open air which dries out the surface. The moist environment ensures a higher protease activity that enables prompt cleaning of wound surface without use of fermentation drugs associated with allergic response. Besides, it prevents dryness of nerve endings and relieves pain. As the dressing does not stick to the wound, there is no pain on removal and no damage to granulation tissue and growing epithelia.

A dominant tendency in the development of modern wound dressings is the use of polymer matrix composites based on biocompatible natural and synthetic polymers. Effective healing of wounds and wound infections requires dressings to have a high sorption activity, effectively removing excessive wound exudate and its toxic components. Such dressings are semi- permeable to vapor and at the same time prevent dryness of the wound surface and protect from secondary infection of the wound; they are elastic and capable of restoring surfaces with complicated relief, and they are mechanically robust. Dressings should provide an optimal microenvironment for wound recovery and cause neither pyrogen and toxic effects nor irritant and allergic reactions.

One of the advantages of dressings based on composite matrices is their ability to deliver antibacterial, antiseptic, anti-inflammatory drugs that facilitate reparative processes. Transparent films allow visualization of the wound bed.

Absorbable membranes made from polymer matrices mostly conform to all clinical specifications and could be applied at all stages of wound and burn treatment. Optimal mechanical and physicochemical properties of hydrogel compositions used in drug delivery enable a prolonged effect of drug substances, targeted drug delivery and possibly a marked synergistic effect of drugs.

Chitosan and its derivatives are viewed as most promising biopolymers. Chitosan is not toxic, it is biocompatible and biodegradable. It has antimicrobial and immunostimulating activities. Chitosan is a hemostatic agent, it accelerates coagulation process, and blocks nerve endings alleviating the pain. Chitosan has been actively investigated as the basis of wound and burn dressings and a major component of multiple products: hydrogels, membranes, films, nanofibers, granules, nanoparticles and scaffolds. Scaffolds are of particular interest as new universal forms which can be utilized in tissue engineering and regenerative medicine.

In the section *Surgery* you will be offered the data on effectiveness of novel developments in chitosan-based dressings used for treatment of biliary and heavy exudate wounds. Results of researches broaden our knowledge on application of chitosan-based wound dressings and their anti-inflammation and regenerative properties.

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TELEMEDICINE AS AN ESSENTIAL PART OF THE MODERN HEALTHCARE DEVELOPMENT

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ABSTRACT — Widespread use of telemedicine as a method for remote medical care provision can become one of the most promising ways to optimise modern healthcare. This article proposes a methodology for adult health telemedicine screening using a system for analysing unstructured data of monitoring chronic non-communicable diseases in the population. This method is based on the risk estimation by the Decision Rules approach, and the fuzzy set theory is used as a description tool. Scientifically-based comprehensive clinical approach for the development of medical questionnaires, the knowledge of which depends on a large scientific base in the field of screening, propaedeutic, taking into account the standards and requirements of health assessment in the Russian Federation, is a fundamental difference from the existing analogues. Expert system of telemedicine questionnaire screening allows determining the health risks by specific profiles, to develop a final personalised judgment with recommendations for a healthy lifestyle, further examination, treatment, and prevention of chronic non-communicable diseases.

KEYWORDS — telemedicine, screening, chronic diseases, health protection, quality improvement in healthcare, information and communication technologies (ICT).

BACKGROUND

Currently, there is a steady increase in public spending related to healthcare worldwide. Widespread introduction of telemedicine in general practice as a method for provision of qualified remote medical care is one of the promising options for optimising such costs.

Telemedicine has existed for a long time one way or another. E.g., in 1905, Einthoven first transmitted ECG records using telephone communication. Since 1922, the University Hospital in Gothenburg has been providing medical consultations to seafarers via radio channels. In 1959, the first television consultation of a psychiatric patient was held in the United States. Later in 1965, the American cardiac surgeon, M. Debeki, consulted surgeons, performing a surgery in Geneva, via a satellite communication channel. In 1999, Ms Gerry Nielsen, engaged as a medical specialist at the Amundsen-Scott Research Station in Antarctica, was diagnosed with and treated for breast cancer through satellite communication, email, and video conferencing. [1].

According to WHO, the term *telemedicine* defines health services provision in conditions where the distance is a critical factor by healthcare professionals, using information and communication technologies (ICTs) to exchange the necessary data for the diagnosis, treatment and prevention of diseases and injuries, conduct research and assessments, as well as to continue the education of health professionals for the improvement of public health and the development of local communities [2]. In turn, the European Commission considers this term more specifically and proposes to construe telemedicine as operational remote access to the services of medical specialists, using ICTs, regardless of the patient location or the way the relevant information is stored. [EHTEL, 2008]. According to the definition of the American Telemedicine Association (ATA), telemedicine is the use of medical information provided by one party to another by electronic means of communication to improve the health of patients. [3] Despite the fact that each institution offers its own interpretation of the term, each of the definitions highlights three main characteristics of telemedicine: health care quality improvement, the use of ICT, and remote access. [4].

In 2010, WHO identified four specific principles of telemedicine [WHO 2010]: provision of clinical support, overcoming geographical barriers and establishing communication between physically distant users, using various ICTs, improvement of public health [2].

SYNCHRONOUS AND ASYNCHRONOUS TELEMEDICINE

Telemedicine, depending on the terms of information transmission and interaction between the persons involved in the process, can be divided into 2 types: synchronous and asynchronous [2]. Asynchro-

nous telemedicine is based on the exchange of pre-recorded data between two or more persons at different periods of time. For example, the sending a clinical case to an expert via email with subsequent doctor's feedback on the diagnosis, optimal therapy, and recommendations for a healthy lifestyle management can serve as the examples of asynchronous telemedicine, Synchronous telemedicine is applicable in real time and requires simultaneous presence of participants in the process, e.g., during video conferences, phone conversations or via the Internet. In both synchronous and asynchronous telemedicine, information can be transmitted in various forms: as text, audio, video, or image [5].

REMOTE MONITORING AND COVID-19

Remote monitoring can be singled out separately. This is a form of telemedicine that involves remote monitoring of the patient's condition by medical professionals using various technical devices. The data obtained during monitoring is subject to remote monitoring and is transmitted to a medical facility for further analysis and interpretation. [4, 6] Remote monitoring is used for dynamic observation of patients with cardiovascular diseases, diabetes mellitus, bronchial asthma, as well as for domiciliary patients monitoring. [3]

The use of modern technologies now makes it possible to provide high-quality medical care without violating the principles of social distancing, which is really important in the context of the COVID-19 pandemic, where telemedicine has become the first line of defence for both patients and medical specialists. [7] As practice has shown, telemedicine can be used in several forms in conditions of global quarantine [7]:

1. Online consultations: tele- and video conferences;
2. Telemonitoring: the use of screening devices, which assess the level of blood pressure, saturation as well as the respiratory rate;
3. Chatbots, where the patient can get answers to frequently asked questions, as well as request connection with the doctor.

Nowadays, telemedicine combines convenience, low cost, and high availability for the end user. As a result, it reduces the time for diagnosis and treatment, including in the context of the COVID-19 pandemic, as well as possibility of close observation without necessity of moving around the city, and direct physical contact with a medical professional, which ultimately reduces the risk of spreading the infection. In addition, telemedicine can be used to control the spread of both non-communicable and infectious diseases, including COVID-19 [8, 9, 10].

Recent events related to the COVID-19 pandemic, forced self-isolation regime, cancellation of face-to-face appointments and re-profiling hospitals have given impulse for development of telemedicine in Russia. The population began to show more and more interest in telemedicine, considering that after the beginning of COVID-19 the need for medical consultations did not disappear, but, on the contrary, increased. Thus, in 41% of cases, a face-to-face consultation was not required after a remote request for medical care, 20% of those who applied were re-admitted to the same specialist and in 30% of cases, and patients were immediately referred to a narrow-profile specialist without another consultation with a therapist [11].

TELEMEDICINE MARKET AND SERVICES

Currently, the development of telemedicine services is an absolute trend in the development of the digital industry worldwide. According to various estimates, the average annual growth rate of recently emerging online medical opportunities for users in the next five years will be about 116%. If the volume of the Russian telemedicine market was estimated at RUB 1.5 bln in 2019, it is expected to grow more than 60 times by 2025, reaching RUB 96 bln, which is consistent with the VEB Ventures opinion. At the same time, the company notes that the growth driver will be not just the service demand dynamics effect, but also the availability of telemedicine platform services for a wide range of patients [12, 14].

In terms of the global market, according to BBC Research, the global telemedicine market increased to an average of $44 bln in 2019. According to P&S Market Research in 2016, its volume did not exceed $18 bln. Lower market activity in developing countries is related to insufficient payback and lack of technological infrastructure. In developed countries, telemedicine complements traditional medicine, while in developing countries telemedicine is often an alternative to traditional medical care. Despite this fact, the emergence of telemedicine in developing countries opens up access to health services for a wide range of people, as well as reduces geographical barriers [12].

We analysed the market of companies, developing telemedicine, and found 35 services, and almost all of them interact with the client by means of a video camera (video service), smartphone, or computer [13–20]. The price of such services in the foreign market ranges from $5 per month to $441 per insurance package per year. For comparison, in 2020, the average check in private clinics in the Russian Federation was amounted

to RUB 3,000 (€33) per visit, and the average cost of a minimum check-up: therapist's appointment, blood and urine tests, lung X-rays and ECG was amounted to RUB 40,000 (€442). The price of a specialist consultation using telemedicine technologies on the Yandex-Health portal starts from RUB 499 (€5.5). The main type of services provided is the patient flow control, remote telemedicine counselling, and signal processing from gadgets [21].

Thus, in terms of cost-effectiveness, it is obvious that the introduction of telemedicine will reduce health care costs [22, 23]. The reduction in health care costs will be achieved by reducing the cost of hospitalisation, patient transportation, and indirect costs associated with leaving home or office [24]. Thus, a number of Russian and foreign studies have shown a reduction in costs for telemedicine in comparison with traditional methods from 2 to 73%. Even now, telemedicine consultations for the end user have become over 10 times more profitable than face-to-face consultations [22, 25].

Thus, there are all the prerequisites for the emergence of own developments using telemedicine technologies in Russia. However, among the well-known programs, along with their undoubted advantages, such as accessibility to the public, possibility of remote examination, relatively low cost of screening, there are also disadvantages such as lack of final analytical documents, coverage of one or more systems, need for a face-to-face visit to the doctor to estimate the risks and determine further tactics of patient control [26].

MATERIALS AND METHODS

We have developed our own version of telemedicine questionnaire screening for adult health, based on the experience of national and foreign colleagues. The scientific-based comprehensive clinical approach to the development of the medical questionnaire, the knowledge of which is based on a large scientific base in the field of screening, propaedeutic, taking into account the standards and requirements of the health assessment of the Russian Federation, is a fundamental difference from the existing analogues. Creation of a decision tree model and development of algorithms for risk share estimation, taking into account external factors, including the specifics of the residence halo, cultural characteristics of nutrition, and others, will improve the quality of medical screening, its reliability, and will also make it possible to give the user optimal recommendations on the strategy of diagnosis and treatment. Over the past year and a half, the authors have done a great deal to create a telemedicine system of questionnaire screening, a methodology for selection and ranking of questions that are most informative, simple and available for stating, taking into account an integrated approach [27, 28].

The study was conducted at the Department of Internal Medicine, Clinical Pharmacology and Nephrology of the North-Western State Medical University named after I.I. Mechnikov, based on our platform for telemedicine medical support. The study involved 139 people: 97 women and 42 men, the average age of the subjects was 65±13.8 years.

We did not use a clinical entity as a health risk assessment in the telemedicine system, but rather a pathology profile, in which a set of analytical features, objective examination data, and complaints were divided into groups. 198 questions were prepared for conducting a multidisciplinary comprehensive questionnaire health screening; these questions were structured according to 5 pathology profiles: cardiology, endocrinology, gastroenterology, pulmonology, and oncology. These pathology profiles were not chosen by chance: Since the second half of the 20th century, the main death causes have been chronic non-communicable diseases (chronic NCDs): circulatory illnesses, chronic bronchopulmonary diseases, oncological diseases, and diabetes mellitus. In the Russian Federation, about 75% of all deaths occur annually due to chronic non-communicable diseases, however, particularly high mortality is observed among people of working age [29]. In addition, the prevalence of digestive diseases has increased in recent years [30].

RESULTS AND DISCUSSION

All the fairly ascertained features of each profile were evaluated according to the degree of their severity or the reliability of their presence. Minimal deviations were taken for early detection of pathology or low risk, in cases of its combination with any complaints or developmental features noted in the questionnaire. The program provides both identification of the severity degree and attribution of the symptom or sign to the pathology of several systems, since they can often appear during destruction of different organs, and therefore the estimation of the disease risk spectrum is carried out by the system in accordance with all five profiles. In turn, the system is configured for the boundary between the risk group and the pathology, estimated at 50 points, and the range of the risk group is concentrated in the range from 20 to 50 points. The risk estimation is carried out by the *Decision Rules* method, and the fuzzy set theory is used as description. The typical features of the mathematical apparatus used are, on the one hand, the ability to formalise ideas about the degree of severity of a particular symptom, and on the other hand, the adequacy of medical logic.

Using the developed technology, it was found that 33.8% of the subjects were diagnosed with a low risk of cardiovascular diseases, 41.7% — an average risk, 24.5% — a high risk. While analysing the answers to the questions of the gastroenterological profile, 33.1% had a low risk of gastrointestinal diseases, 46.8% had an average risk, and 20.1% had a high risk. 46.7% of the subjects had a low risk according to the *Pulmonology* profile, 40.3% had an average risk, and 13% had a high risk. 6.5% of the respondents were diagnosed with low risk in the *Endocrinology* profile, 23.7% were diagnosed with medium risk, and 69.8% were diagnosed with high risk, however, this profile requires further development due to the non-specificity of endocrinological complaints. 18% of the subjects have a low risk of cancer, 32.4% have an average risk, and 49.6% have a high risk of cancer, according to the questionnaires, which requires increased cancer alertness in this study group. The average score of the subjects in the cardiological profile was 517, in the gastroenterological profile — 440, the average value in the *Pulmonology* profile was 439, the average value in the *Endocrinology* profile was 495, and the average value in the *Oncology* profile was 533. 12 subjects (8.6%) were revealed to have a high risk in all 5 pathology profiles. And only 7 (5%) of the examined patients were diagnosed with a low risk for all pathology profiles.

The program analysed the obtained data and offered appropriate recommendations depending on the age, gender, body mass index, vicious habits, and level of physical activity and identified risk factors of the subject. Thus, in case of a low risk of disease development in all 5 pathology profiles, it is recommended to follow healthy lifestyle rules, including the principles of proper nutrition, body weight control, sufficient physical activity, rejection of vicious habits and medical examinations according to the established deadlines. In case of an average risk of disease development, depending on the pathology profile, in addition to the healthy lifestyle, it is recommended to monitor blood pressure daily, as well as to determine a set of necessary laboratory and instrumental examinations, and immunisation. In case of high risk of pathologies, the program recommends a full-time consultation with a doctor to determine the further strategy of examination and treatment of such a patient, as well as a dispensary follow-up with an appropriate specialist at the place of residence.

CONCLUSION

Thus, our program makes it possible to determine the degree of existing health risk by specific profiles (cardiology, gastroenterology, pulmonology, endocrinology, oncology), to develop a final judgment and recommendations for a healthy lifestyle, further examination, treatment and prevention of chronic diseases.

The innovation of this development lies in in-house methodology for the selection and ranking of clinical symptoms and signs of diseases, taking into account their severity and reliability. The specified symptoms and signs of diseases create not a clinical entity, but a pathology profile, and therefore they have the greatest information content, statement availability, and cover all the key body systems. Moreover, an original case of summary documents with recommendations on lifestyle changes has been developed.

The benefits of this development are the flexibility of the decision rules, corresponding to the opinion of the expert doctor, the convenience and clarity of the representation of the final results, the formulation of conclusions on the necessary follow-up and recommendations for adherence to a healthy lifestyle, as well as individualised approach, mobility, absence of reference to time and place, and the availability of *doctor–patient* feedback.

Acknowledgment

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ISOLATION AND PURIFICATION OF PROTEIN THAT IS IMMUNOLOGICALLY SIMILAR TO HUMAN LACTOFERRIN

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ABSTRACT — The study aimed at isolating a substance that is immunologically similar to human lactoferrin, hereinafter — microbial-derived lactoferrin (MdLF) isolated from *K.pneumoniae* liquid culture.
Protein extraction started with isolation of ballast proteins in 2M ammonium sulphate (p.a.). Ion chromatography with cation-exchange agents was the basic method used for isolating MLF. Proteins from the isolated protein fractions were identified with AGID method. The isolated MLF is glucoprotein with the molecular mass of about 84,000, the agarose diffusion coefficient of 3.1×10^{-7} cm^2 sec^{-1} and the relative electrophoretic mobility of 0.42. MLF is characterized by low hydrophobicity and elutes from phenyl-Sepharose with 0.4 ammonium sulphate. Its isoelectric point equals to 9.2.

KEYWORDS — microbial-derived lactoferrin, chromatography, electrophoresis.

RELEVANCE

Lactoferrin belongs to the transferrin family and is capable of active binding and transporting of Fe^{3+} and other mixed-valent metals. Previously examined lacroferrins are glycoproteins with the molecular masses within the range of 75–80 kDa, containing intramolecular disulfide bonds. A polypeptide chain of lactoferrin forms two globular domains called M- and C-lobes, connected with an alpha helix. Each lobe has an iron-binding centre. The tertiary structures of apo-lactoferrin (iron-free lactoferrin) and iron-binding lactoferrin differ [4, 6, 7, 8, 9, 10, 13]. For example, it is found that Fe^{3+} binding plays an essential role in implementing the antioxidant and bacteriostatic function of lactoferrin. Along with antimicrobial activity, lactoferrin also has other biological properties — it can act as an immunomodulatory agent, antioxidant and anti-inflammatory agent and transcriptional factor and definitely participates in iron metabolism [1, 5, 14, 15, 17].

Comparative studies play a significant role in examining biological properties of lactoferrin, for which reason it is important to examine the structure and properties of lactoferrin from the sources covering different species. Interestingly, literature sources still contain only sporadic data on the presence of lactoferrin in prokaryotes with no protein isolated and no molecular mass measured [2, 3, 10, 11, 12].

Anyway, microbial cells have been increasingly used as potent protein factories because of their versatility and cost-effectiveness [18].

We have made an attempt to isolate and purify a protein that is immunologically similar to human lactoferrin from Klebsiella pneumoniae. MdLF presence in the samples of the pre-centrifuged (5,000 rpm during 45 minutes) liquid culture of *K.pneumoniae* (meat-peptone broth, incubation time — 24 hours at t=37° C) was assumed on the basis of EIA results with the use of *Hycult biotech* test kits (Netherlands) [3].

MATERIALS AND METHODS

Currently, lactoferrin is commonly isolated by the method of ion chromatography with cation-exchange agents. This method is based on a significant difference of the LF isoelectric point from other proteins at the pH values of 7–7.4.

Protein extraction started from isolation of ballast proteins in 2M ammonium sulphate (p.a.). MLF does not precipitate at this concentration, and once a precipitate was centrifugally isolated (10,000 rpm during 45 minutes), it was resuspended in a buffer (10 mM tris-HCl, pH 7.6; 1 mM EDTA; 1 mM iron(III)-ammonium) to saturate LFP with iron.

After this, the sample is thoroughly desalted and buffered with a phosphate buffer, pH=7.0. Then the obtained solution was applied on a column (4.5×60 cm) of Heparin Sepharose (Sigma, USA) which had been preliminarily equilibrated with 20 mM tris-HCl, pH 7.5. The sorbent was washed with 20 mM tris-HCl, pH 7.5, and then — with the same buffer, containing 1% triton X-100, and then again – with the buffer with no triton until the optical absorption disappeared. Elution was carried out with NaCl linear gradient with the concentrations from 0 to 1 M

in 20 mM tris-HCl, pH 7.5. The obtained fractions were dialyzed against 10 mM tris-HCl, pH 7.5 at 4° C during 16 hours.

Unlike the general method of isolating MdLF, the heparin sepharose column had been preliminary treated with 0.2–0.3% formaldehyde solution in order to oxidize minor functional groups of heparin, which leads to an increase in its negative charge and, as a result, increases it affinity in relation to lactoferrin.

The molecular mass of lactoferrin was measured with DC-Na-polyacrylamide gel electrophoresis according to Schägger and von Jagow's method with the use of 8% acrylamide gel. The molecular mass was also evaluated upon gel filtration results.

RESULTS

The isolated MdLF is glucoprotein with the molecular mass of about 84,000, the agarose diffusion coefficient of 3.1×10^{-7} cm^2 sec^{-1} and the relative electrophoretic mobility of 0.42. LFP is characterized by low hydrophobicity and elutes from phenyl-Sepharose with 0.4 ammonium sulphate. Its isoelectric point equals to 9.2.

Despite its immunochemical identity with human milk lactoferrin, MdLF differs from lactoferrin by some of its physiochemical parameters. The difference is especially evident in the electrophoretic mobility of MdLF and human milk lactoferrin. According to the obtained data, the MdLF relative electrophoretic mobility is 0.41±0.006, while the one of human milk lactoferrin is 0.47±0.003. These differences may be considered another evidence of the presence of several isoforms of lactoferrin that have the same molecular mass and are immunochemically similar but differ in their affinity to different ionogenic groups.

Dissociation of the LF and Fe^{3+}complex was examined with two methods — at low concentrations of chelating anions (Pe^ dissociation synchronically happens from two iron-binding centres of LF) and at high concentrations of chelating anions (Fe^{3+} dissociation from two iron-binding centres happens separately). Iron-saturated LF (chololactoferrin) has a typical visible absorption spectrum. The spectra appeared to be similar for the bacteria and human proteins. No difference in the bacteria and human proteins was identified in the experiments on the LF and Fe^{3+} complex dissociation. Summary data on the MdLF physicochemical properties are presented in Table 1.

Table 1. MdLF physicochemical properties

Properties	LFP
Molecular mass	85,000±5,000
Relative electrophoretic mobility in agar	0.42±0.005
Agarose diffusion coefficient, cm^2 sec^{-1}	3.1×10^{-7}
Hydrophobicity	Elution from phenyl-Sepharose with 0.4 ammonium sulphate
Precipitation with ammonium sulphate, % saturation and precipitation range	50–75
Full precipitation	75
Precipitation with 50% ethanol	No precipitation
Precipitation with 60% acetone	Full (reversible)
Precipitation with 0.9M chloric acid	Irreversible
Isoelectric point	9.2
Thermal resistance	Resistant at 65° C — 30 minutes

CONCLUSION

Modern research allows to state that iron is a universal factor of microbial growth. Being in the environment, iron is exposed to oxidation and hydrolysis processes, leading to a decrease in the concentration of Fe^{2+} and Fe^{3+} free ions down to 10^{-9}–10^{-18} M, which is not sufficient for optimal vital activity of most of the microorganisms. Likewise, iron is bound to iron-binding proteins in the mammal organisms and is unavailable for microorganisms. That is why microorganisms have evolved ways of getting iron in iron-deficiency conditions, which is important for optimal development in environment and, in certain cases, may be critical for survival of the population. In this case, microbial strategies for getting iron, including MLF synthesis, can be considered as one of the factors that determine the formation and stability of microbial associations, playing an important role in the functioning of normal and pathological microbiocenoses in the host.

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PERSONALIZED FSI-MODELING OF THE AORTIC BULB AND ARCH TO PREDICT ITS MECHANICAL BEHAVIOR AND ASSESS THE LOADS DURING THE CARDIAC CYCLE

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ABSTRACT — The interaction of the blood flow with the aorta is a complex dynamic event described in biomechanics as the Fluid-structure interaction. In this study we've developed a method for creation of a personalized 3D dynamic model of the aortic bulb and arch for the prediction of its mechanical behavior using FSI-analysis. We found that the accuracy of predicting geometric aortic deformities based on FSI modeling is on average 92%.

KEYWORDS — aorta, aortic valve, hemodynamics, FSI-modeling.

RELEVANCE

The interaction of the blood flow with the aorta is a complex dynamic event described in biomechanics as the interaction of a fluid and a deformable body (Fluid-structure interaction or FSI). The existing methods of in vivo visualization and quantitative analysis in silico allow us to model these processes in order to study the pathogenesis of diseases of the cardiovascular system, predict risks and plan surgical interventions [1]. The FSI method is widely used for numerical modeling of the blood-vessel interaction [2, 3, 4]. It combines the methods of computational fluid dynamics and structural (dynamic) analysis. Today, FSI is widely used in predicting the risks of aneurysms and their ruptures. Previously, no attempts have been made to study changes in the biomechanical properties of the aorta in aortic valve stenosis using personalized FSI modeling based on MSCT data.

Aim: to create a personalized FSI model of the patient's aortic segment and then to evaluate its mechanical behavior during the cardiac cycle.

MATERIALS AND METHODS

Functional MSCT-coronarography data were used to model the aortic segment. Based on these data, in the InobitekDicom Viewer software, a multiplanar reconstruction of the zone of interest was generated, including the anatomical structures of the heart and aorta. For the aortic valve, the ascending aorta, and the aortic arch, routine segmentation was performed in three projections. From the resulting set of contours, a three-dimensional model was created, which was then converted into a polygonal STL model (Fig. 1).

Fig. 1. *STL model of the zone of interest*

Editing and reverse engineering of the STL model was performed in the SolidWorks software. The aortic wall and its root were generated using the SW ScanTo3D utility. The valves and commissures of the aortic valve were modeled using standard SW tools.

The CAD model of the zone includes the structures of the aorta, the fibrous ring, the valves, commissures and sinuses of the Valsalva (Fig. 2).

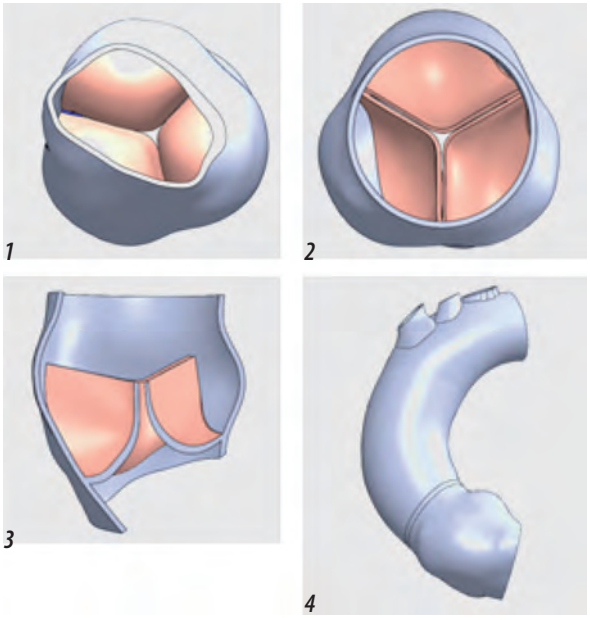

Fig. 2. Aortic valve model: 1 — bottom view, 2 — top view, 3 — split view, 4 — full CAD model

Preprocessing of the CAD model for FSI analysis was performed in HyperMesh software. The calculated KE-grid was generated taking into account the anatomical and morphological features of the aortic wall.

The resulting CE model was imported as an orphan grid in Abaqus CAE to set the mechanical properties of the materials and set the boundary conditions for the biomechanical analysis of the model. To optimize the computational load, we introduced the following boundary conditions: the model is fixed (Fig. 3) at the entrance (the plane of the sinotubular junction of the aorta) and at the exit of the blood flow (in the area of the brachiocephalic trunk, the left common carotid artery and the left subclavian artery) in 4 nodes located in the centers of the above areas.

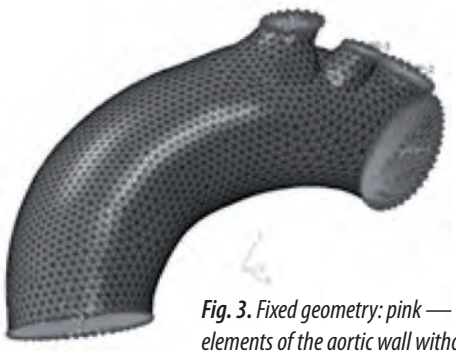

Fig. 3. Fixed geometry: pink — connected elements of the aortic wall without restrictions of degrees of freedom, the intersection of yellow lines — a node with a fixed geometry

Each node is connected by the condition of the continuum distribution with the elements of the aortic wall in the planes of sections at the entrance and exit, without limiting the degrees of freedom of the connected elements. In the area of the border of the arch and the descending part of the aorta, the model is fixed in the area of the arterial ligament with the condition of the distribution of the continuum with the elements of the aortic wall in the cross-sectional plane without limiting the degrees of freedom of the connected elements.

The blood flow is specified as laminar, the flow rate at the peak of the systole is v = 1.2 m/s (Fig. 4) [5], the flow input is the entire area in the plane of the sinotubular junction of the aorta, the output is the areas of the brachiocephalic trunk, the left common carotid artery, the left subclavian artery, the border of the arch and the descending aorta (outlet pressure P = 0).

The connection condition (FSI) between the aorta and the fluid was set by the Fluid-Structure Co-simulation boundary parameter. This parameter describes the interaction between the Computational Fluid Dynamics (CFD) module and the Abaqus CAE Implicit Dynamic Analysis (Dynamic/Implicit) module. The choice of the Dynamic/Implicit module is based on the hypothesis of small deformations in the simulated segment, which eliminates the need to use adaptive meshes.

For the subsequent formulation of the FSI problem, the flow region was modeled. The final grid of finite elements for aortic structures consists of 32109 tetrahedral elements, for the flow region (blood) — 34999 tetrahedral elements. To model the anisotropic properties of aortic structures, we used the Holzapfel-Gasser-Ogden (HGO) anisotropic material model. To evaluate the proposed model, an FSI analysis of the aortic segment, including the ascending section and the aortic arch, was performed during systole (normal). The results of the analysis were compared with the MSCT data and analyzed by the expert control method. In this paper, the model adequacy criteria were taken as the deformed states of the FSI model, which were compared with the displacements on the MSCT at the corresponding moments of the systole. Aortic valve stenosis was modeled by changing the area and peak flow rate during systole at the model inlet.

RESULTS

For the validation model, Mises displacement, pressure, and stress plots were obtained for the aortic

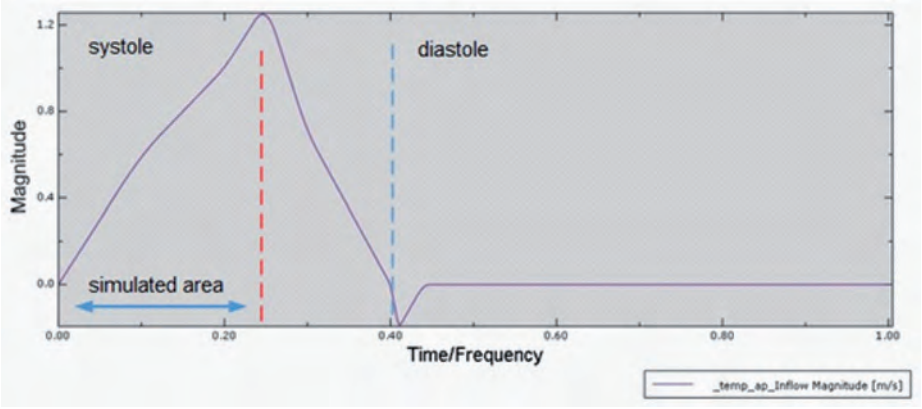

Fig. 4. Graph of changes in the flow rate during the cardiac cycle. The simulated area is 0.23 seconds from the beginning to the peak of the systole

wall. Changes in the blood flow characteristics in the model are described. All initial conditions were modeled for systole at time t = 0 in accordance with the MSCT data at the same time. The FSI analysis made it possible to predict the behavior of the aortic wall during a systole lasting 0.23 seconds. The prediction result (displacement) was compared with the MSCT at time t = 0.1 s, and t = 0.23 c corresponding to the peak of systole (Fig. 5).

the 3D reconstruction of the MSCT for the corresponding time points. The overlay of each STL-grid was carried out by means of rigid registration of images based on the intensity (Fig. 6).

The IoU coefficient for the volume model was calculated as the arithmetic mean IoU of all 2D images. The maximum value of the coefficient for the two forecasts of the FSI model is 0.96, and the minimum value is 0.73. The average IoU value for the two models

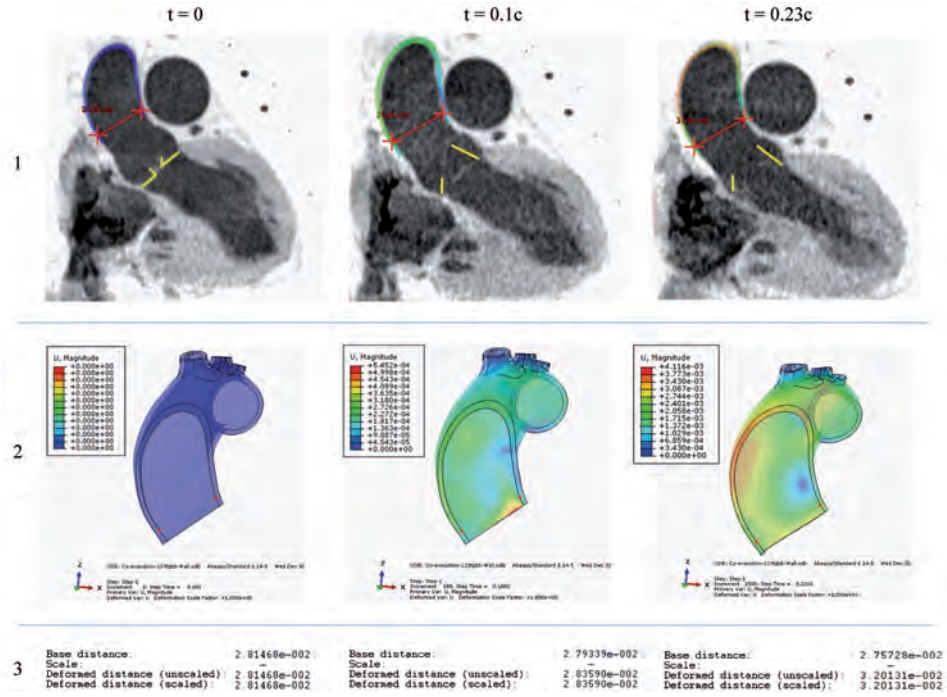

Fig. 5. Plots of movements in meters (line 2) at the corresponding moment

We investigated the geometric deviations of the FSI simulation from the MSCT data by superimposing and analyzing the STL meshes extracted from the solver output file at time t = 0, t = 0.1 c, t = 0.23 c, on

was 0.92. Figure 7 shows the Mises stress plots (1), the pressure distribution along the aortic wall (2), and the flow characteristics at the peak of systole t = 0.23 s (3) in normal, predicted by FSI modeling.

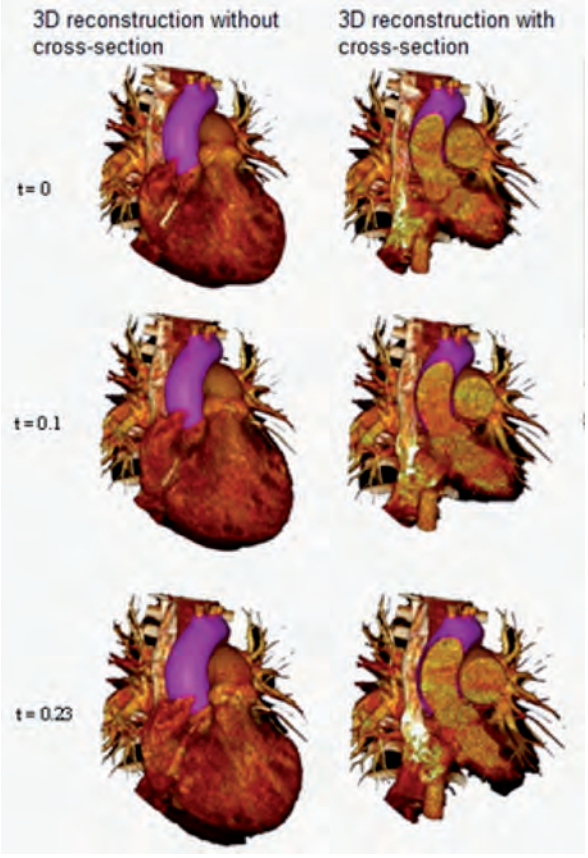

Fig. 6. *MSCT-FSI combined 3D reconstructions. The deformed STL meshes are highlighted in pink. The anatomical structures of MSCT reconstruction are encoded by a soft tissue imaging filter*

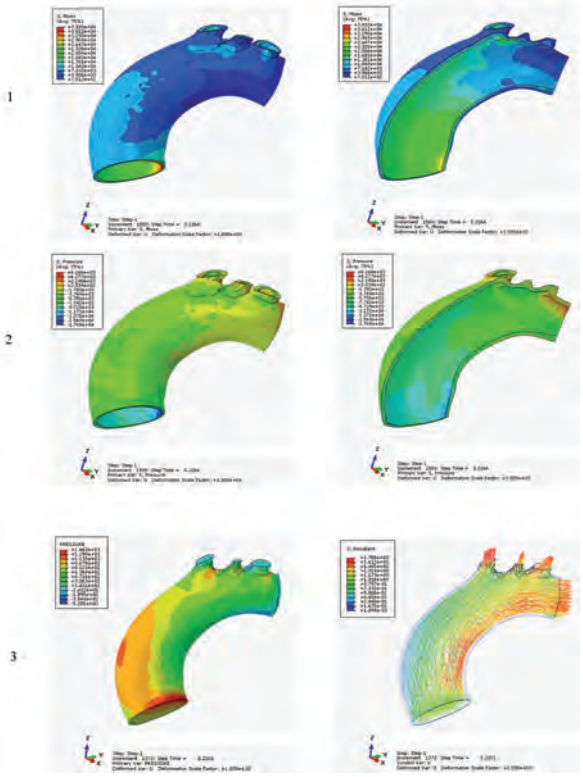

Fig. 7. *Prediction of hemodynamics and mechanical behavior of the aorta in normal conditions*

CONCLUSION

We established that the accuracy of predicting geometric aortic deformities based on FSI modeling is on average 92%. The results of modeling using the proposed methods do not contradict the previously obtained data in silico. The predicted deformed states correspond well to the in vivo data.

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EFFECT OF LEAD ON STRUCTURAL CHANGES IN LIVER OF WISTAR RATS UNDER THE CONDITIONS OF ACUTE EXPERIMENT

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ABSTRACT — The purpose of this study was to evaluate the structural transformations of Wistar rats liver after oral administration of lead salts. Under the conditions of acute experiment during 5 days the Wistar rats were orally administered the lead acetate solution in the amount of 3 mg/kg. Histological studies were carried out at OSMU upon the completion of the experiment. Effect of toxic doses of lead causes symptoms of both steatosis and hydropic degeneration of the liver. It is assumed that the appearance of hepatic steatosis reflects the reaction of hepatocytes to hemic hypoxia caused by the action of lead, while the signs of hydropic degeneration expressed in varying degrees reflect its direct toxic effect.

KEYWORDS — lead, liver, hepatocytes, hydropic degeneration, hepatic steatosis.

RELEVANCE

In recent decades, heavy metals are increasingly frequently named as the most dangerous environmental pollutants. Their migration and redistribution in the components of ecosystems depend both on the whole complex of natural factors, and the intensity and nature of technogenesis. Lead is not a vital element. It is toxic and belongs to danger class I. Its inorganic compounds interfere with the metabolism and are the enzymes inhibitors (like most of the heavy metals). One of the most insidious consequences of the action of inorganic lead compounds is its ability to accumulate in a number of organs, including the liver, and to be a constant source of poisoning for a long time. The main source from which lead enters the body, is the food, and also the inhaled air plays the important role. Absorption in the gastrointestinal tract is up to a 5–10% in adults, and 50% in children. Thus, there is a need to assess the toxic effect of lead.

The purpose of this study:
to evaluate the structural transformations in the liver of Wistar rats after oral administration of lead salts.

METHODS

20 male Wistar rats aged 4–5 months, weighing 200–220 g were divided into an experimental and control group — 10 rats in each group. The rats were maintained in standard cages under regular daily alternation of light and darkness, temperature 20–22° C and free access to water and food. The studies were carried out in in compliance with the CIOMS-ICLAS International Guiding Principles for Biomedical Research Involving Animals.

Under the conditions of acute experiment during 5 days the Wistar rats were orally administered the lead acetate solution in the amount of 3 mg/kg. Histological studies were carried out at OSMU upon the completion of the experiment. The cut and oriented liver acinuses were fixed in 10% neutral buffered formalin (pH = 7.2–7.4). They were dehydrated and embedded in paraffin according to conventional technique. 4–5 microns thick paraffin sections were stained with hematoxylin and eosin. Ziehl-Neelsen method (detection of fuchsinophile acid resistant intranuclear inclusions) by Lily's formulation (1969) was used to identify lead pellets in a complex with protein deposits in the form of intranuclear inclusions in the cells of renal tubule. Sections were stained with carbol fuchsin (dissolve 25 g of phenol in 50 ml alcohol, then in 5 g of basic fuchsin, fill with distilled water to 500 ml) over a fire until the vapor (4–5 minutes), then the glass was cooled down and colorant drained. Rinsing was performed in running water with differentiation in a 5% solution of sulfuric acid, followed by counterstaining with methylene blue. Pearls reaction was used to identify hemosiderin.

RESULTS

The following data were obtained after the analysis of liver micropreparations of the experimental animals. During an acute experiment in lead poisoning conditions, the signs of hydropic degeneration in the hepatic tissue were observed from the very first day of the experiment. Predominant changes were observed in centrolobular localization hepatocytes, while

proteinosis, recognizable in the light microscope by the irregular cytoplasm (crumpled up paper symptom) was represented unevenly, in the form of peculiar nests. Plethora of central veins and expansion of adjacent sinusoids were observed, and the said changes were already significantly expressed at day 3 or more of the experiment (fig. 1).

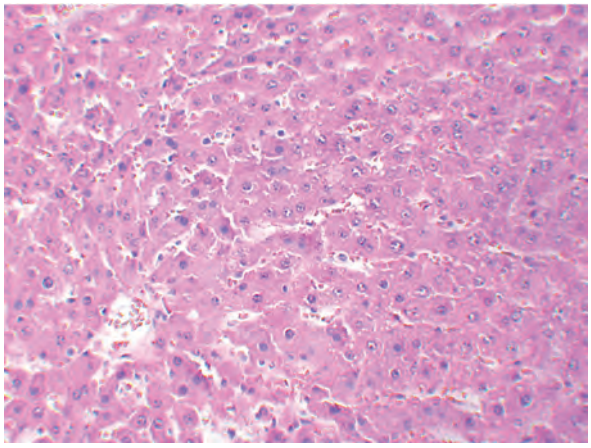

Fig. 1. *Lead poisoning. Dilatation and congestion of central segments of sinusoids. Haematoxylin and eosin staining. ×270*

Signs of proteinosis were characterized by a greater area of distribution covering even the peripheral parts of the acinus. The dystrophy in some hepatocytes of centrolobular localization was expressed up to a balloon type, with the cells with signs of colliquative necrosis (fig. 2).

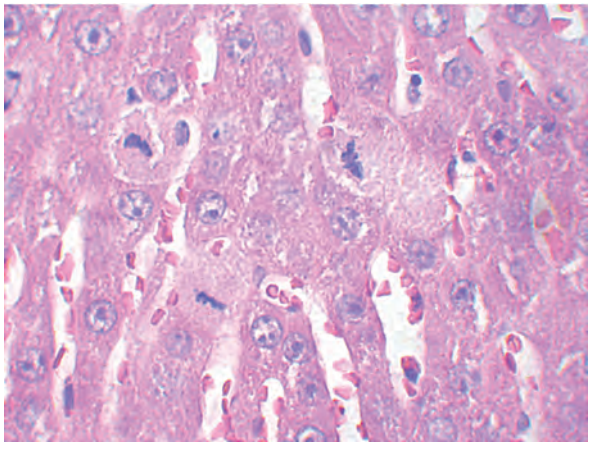

Fig. 2. *Acute lead poisoning. Dilatation and congestion of sinusoids. Coagulative necrosis of hepatocytes, mitoses. Haematoxylin and eosin staining. ×900*

The lumen of the sinusoids and perisinusoidal space was marked with the appearance of hepatic macrophages — Kupffer cells as a rule arranged in groups up to 5–8 cells (fig. 3).

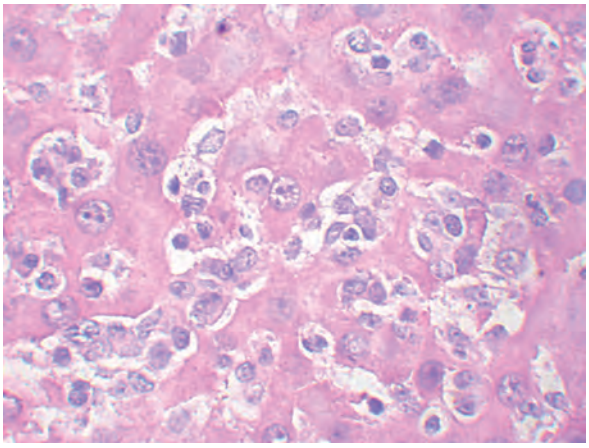

Fig. 3. *Hepatic tissue amid acute experiment of lead poisoning. Clustering of Kupffer cells in congested dilated sinusoids, regenerative transformation of hepatocytes. Haematoxylin and eosin staining. ×900*

By the end of the experiment the majority of the formulations had the signs of not only proteinosis, but also steatosis with small-drop character and represented diffusely in liver acinus (fig. 4).

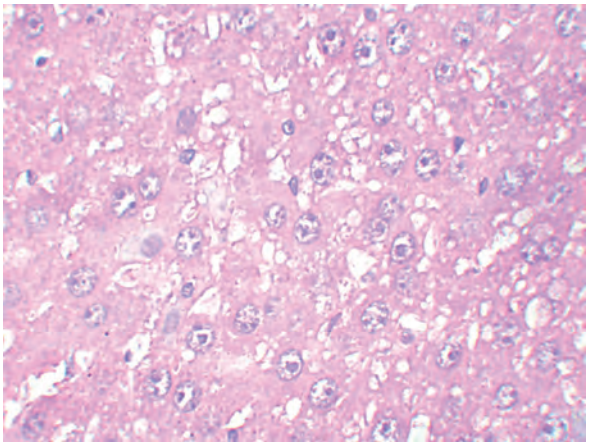

Fig.4. *Lead poisoning. Disseminated small-droplet fatty degeneration of hepatocytes. Coagulative necrosis of certain cells. Haematoxylin and eosin staining. ×900*

Hepatocytes with regenerative changes, hyperchronia of cores with increased size, appearance of binuclear hepatocytes were also observed, mitosis was rarely observed.

CONCLUSION

The effect of toxic doses of lead results in symptoms of both hepatic steatosis and hydropic degeneration of the liver. It is assumed that the appearance of steatosis reflects the reaction of hepatocytes to hemic hypoxia caused by the action of lead, while the signs of hydropic degeneration is expressed in varying degrees reflect its direct toxic effect.

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INFLUENCE OF COPPER COMPLEXES $[Cu(PTA)_4[BF_4]$ AND $Cu(II)_2(3,5\text{-}DIPS)_4(H_2O)_3$ ON THE ORGANISM OF RATS IRRADIATED WITH RADIOISOTOPE TECHNETIUM

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ABSTRACT — One of the priority tasks of modern radiobiology is the search for new, effective radioprotective compounds. In this area, metal-organic complexes with high antioxidant activity are of particular interest.
In order to study the possible beneficial radioprotective effect of copper complex compounds $[Cu(PTA)_4[BF_4]$ and $Cu(II)_2(3,5\text{-}DIPS)_4(H_2O)_3$ on an irradiated organism, we studied cytogenetic parameters in 4 groups of experimental animals: intact animals, animals exposed to the radioisotope technetium (Tc) — "pure irradiation", animals with "irradiation + compound $[Cu(PTA)_4[BF_4]$", and animals with "irradiation + compound $[Cu(II)_2($ 3,5-DIPS$)_4(H_2O)_3$".
The survival rate and cytogenetic parameters were studied: mitotic index (MI), chromosomal aberrations (CA) and polyploid cells (PC) in the bone marrow cells of the femur. The survival rate in the first and third groups was 100%, in the second group — 40%, and in the fourth — 80%. The dynamics of survival was described by regression curves and equations, which make it possible, using extrapolation, to determine the change in the percentage of survival in the long term of the experiment and to predict the further outcome of the experiment.
When analyzing the results in 4 groups of animals, we found a significant difference in cytogenetic parameters between these groups. Thus, for all 3 indicators, a significant difference is observed between intact and irradiated animals, i.e. these parameters can be considered as markers of Tc exposure. In terms of the mitotic index (proliferative activity), a significant difference was found in the irradiated compared with the groups: "irradiation + $[Cu(PTA)_4[BF_4]$" and "irradiation + $Cu(II)_2(3,5\text{-}DIPS)_4(H_2O)_3$", which indicates the radioprotective property of both compounds. By the number of polyploid cells, a significant difference was found between the groups: "pure irradiation" and "irradiation + $Cu(II)_2(3,5\text{-}DIPS)_4(H_2O)_3$", which also proves the beneficial effect of this compound.
Multiregression analysis of cytogenetic parameters along with standard statistical methods confirmed the highest efficiency of $[Cu(PTA)_4[BF4]$ relative to $Cu(II)_2(3,5\text{-}DIPS)_4(H_2O)_3$.

KEYWORDS — radiation, chromosomal aberrations, polyploid cells, mitotic index, survival.

INTRODUCTION

It is known that the main initiating event after irradiation of an organism is DNA damage, on the basis of which chromosome destabilization is considered one of the first and direct signs of the effect of ionizing radiation (IR) on a cell [1–4]. Radiation-induced damage to the karyotype is an important indicator both for biological indication of the severity of radiation injuries and for predicting the development of long-term adverse effects of IR.

One of the priority tasks of modern radiobiology is the search for new, effective radioprotective compounds. In this area, metal-organic complexes with high antioxidant activity are of particular interest.

The ability to protect the body from the damaging effects of ionizing radiation in such complexes were noted both in the scientific works of other authors [5, 6] and in our early works [7–10].

Aim: to study the possible beneficial radioprotective effect of copper complex compounds $[Cu(PTA)_4[BF_4]$ and $Cu(II)_2(3,5\text{-}DIPS)_4(H_2O)_3$ on an irradiated organism using cytogenetic parameters.

MATERIALS AND METHODS

In order to study the possible beneficial radioprotective effect of copper complex compounds $[Cu(PTA)_4[BF_4]$ and $Cu(II)_2(3,5\text{-}DIPS)_4(H_2O)_3$ on an irradiated organism, we studied cytogenetic parameters in 4 groups of experimental animals (white, outbred, sexually mature male rats with an average weight of 180 g,; 10 rats in each group).

The animals used in our research were maintained in compliance with European Union Legislation (Directive 2010/63/EU, amended by Regulation (EU) 2019/1010).

The first group included intact animals. The second group consisted of animals exposed to the radioi-

sotope technetium (Tc), which were injected intraperitoneally with an isotope with an activity of 4.8 mCi in a volume of 2 ml — "pure irradiation". The third group consisted of animals that were injected intraperitoneally with a copper complex $[Cu(PTA)_4[BF_4]$ at a dose of 50 mg/kg in a volume of 2 ml one hour before the administration of the Tc isotope («irradiation + copper compound $[Cu (PTA)_4[BF_4]$»). The fourth group involved animals that, before irradiation, received the compound $Cu(II)_2(3,5\text{-DIPS})_4(H_2O)_3$ at a dose of 50 mg/ kg in a volume of 1 ml.

We studied the survival rate and cytogenetic parameters at the metaphase stage of the mitotic cycle (according to the Ford-Wallam method [11], the following were determined: the mitotic index (MI), chromosomal aberrations (CA) and polyploid cells (PC) in the bone marrow cells of the femur (counting in 1000 cells in each). The cytogenetic examination included the analysis of chromosomes using a J.B. Carnoy fixative and with Giemsa stain. The obtained cytogenetic preparations were analyzed according to the generally accepted method of cytogenetic analysis of bone marrow cells (BMC) of white rats according to G. McGregor [12] under a microscope at 900–1400 times magnification.

Data analysis was carried out using a number of specialized statistical packages: Statsoft and SPSS-10.0. We used regression, multi-regression and correlation methods of analysis [13, 14].

RESULTS

The experiment lasted 30 days. The survival rate of the second group was 40%. In the third group, with the injection of the compound [Cu(PTA)4[BF4], the survival rate was 100%, and in the fourth — 80%. The dynamics of survival was described by regression curves and equations (where y_1 is survival under pure irradiation, y_2 is when «irradiated + injection of $Cu(II)_2(3,5\text{-DIPS})_4(H_2O)_3$» and y_3 is when «irradiated + injection $[Cu(PTA)_4[BF_4]$)» shown in Fig. 1, which make it possible, using extrapolation, to determine the change in the percentage of survival in the long term of the experiment and to predict the further outcome of the experiment.

Analyzing the karyotype and proliferative activity of the above cells, we obtained the cytogenetic indicators of these groups, the results of which are shown in the Table 1. Only reliable values of changes in cytogenetic parameters are given.

The types of karyotype disorders (polyploid cell and double fragment) detected after irradiation of animals with technetium are shown in Fig. 2.

When analyzing the results of the animal groups "pure irradiation", "irradiation + $[Cu(PTA)_4[BF_4]$" and

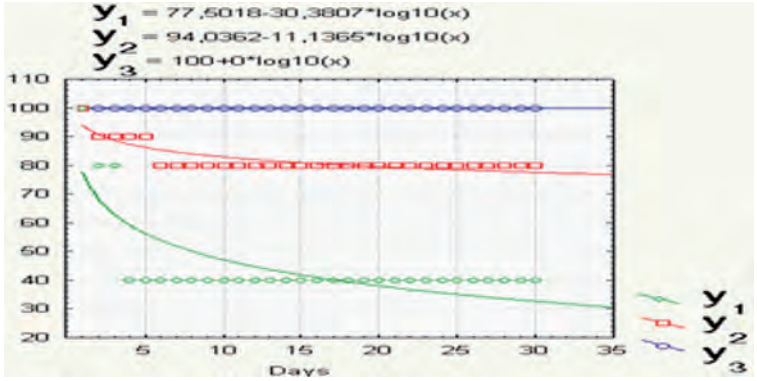

Fig. 1. *Survival of the groups "pure irradiation", "irradiation + $[Cu(PTA)_4[BF_4]$" and "irradiation + $Cu(II)_2(3,5\text{-DIPS})_4(H_2O)_3$"*

"irradiation + $Cu(II)_2(3,5\text{-DIPS})_4(H_2O)_3$", we found a significant difference in cytogenetic parameters between these groups. Thus, for all 3 indicators, a significant difference is observed between intact and irradiated animals, i.e. these parameters can be considered as markers of Tc exposure. In terms of the mitotic index (proliferative activity), a significant difference was found in the irradiated compared with the groups: "irradiation + $[Cu(PTA)_4[BF_4]$" and "irradiation + $Cu(II)_2(3,5\text{-DIPS})_4(H_2O)_3$", which indicates the radioprotective property of both compounds. According to the number of polyploid cells, a significant difference was found between the groups: "pure irradiation" and "irradiation + $Cu(II)_2(3,5\text{-DIPS})_4(H_2O)_3$", which also proves the beneficial effect of this compound.

Fig. 3 shows the results of multi-regression dependences of the mutual influence of cytogenetic parameters upon injection of $[Cu(PTA)_4[BF_4]$ (a) and $Cu(II)_2(3,5\text{-DIPS})_4(H_2O)_3$ (b). In the formulas shown in the upper part of the figure (a) and (b) x is the coefficient characterizing the change in cytogenetic parameters under normal conditions, y is under the influence of Technetium, z1 is under the influence of Tc + $[Cu(PTA)_4[BF_4]$ (a) , and z_2 — under the influence of $Cu(II)_2(3,5\text{-DIPS})_4(H_2O)_3$ (b). Multiregression analysis of cytogenetic parameters along with standard statistical methods confirmed the highest efficiency of $[Cu(PTA)_4[BF_4]$ relative to $Cu(II)_2(3,5\text{-DIPS})_4(H_2O)_3$.

The research results indicate the need to continue work in the direction of searching for drugs that have a therapeutic effect in radiation injuries.

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Table 1. *Cytogenetic indices in 4 groups: "normal", "pure irradiation", "irradiation + $[Cu(PTA)_4[BF_4]$" and "irradiation + $Cu(II)_2(3,5\text{-}DIPS)_4(H_2O)_3$" on the 30th day of the experiment*

groups / indicators	Norm (group 1)	Tc (group 2)	Tc+ $[Cu(PTA)_4[BF_4]$ (group 3)	Tc+ $Cu(II)_2(3,5\text{-}DIPS)_4(H_2O)_3$ (group 4)
Mitotic index (MI) ‰	20,1±2,8	10,9±0,35 $0,01<p_{n2}<0,02$	14,2±0,96 $0,01<p_{23}<0,02$	17,2±1,9 $0,002<p_{24}<0,01$
Chromosomal aberrations (ChA) %	3,0±0,22	6,2±0,5 $p_{n2}<0,001$	4,8±0,42 $0,002<p_{n3}<0,01$	4,8±0,5 $0,002<p_{n4}<0,01$
Polyploid cells (PC) %	0.001±0.0001	3,5±0,44 $p_{n2}<0,05$	3,4±0,47 $p_{n3}<0,05$	2,0±0,2 $p_{n4}<0,05$ $0.01<p_{24}<0.02$

p_{n2} — when comparing the indicators of the second group and the group of intact animals
p_{n3} — when comparing the indicators of the third group and the group of intact animals
p_{n4} — when comparing the indicators of the fourth group and the group of intact animals
p_{23} — when comparing the indicators of the second and third groups
p_{24} — when comparing the indicators of the second and fourth groups

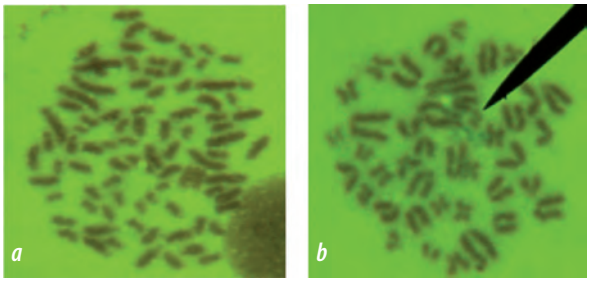

Fig. 2. *Polyploid cell (a) and double fragment (b) 45 days after exposure to technetium isotope*

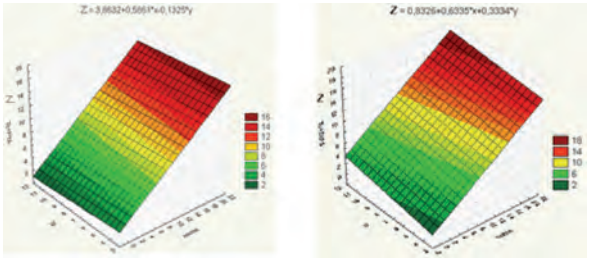

Fig. 3. *Interdependence of cytogenetic parameters upon injection of $[Cu(PTA)_4[BF_4]$ (a) and $Cu(II)_2(3,5\text{-}DIPS)_4(H_2O)_3$ (b)*

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EVALUATION OF THE CORTICOSTEROID RECEPTORS' LEVEL IN THE KIDNEYS OF RATS AFTER SYSTEMIC ISCHEMIA-REPERFUSION

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ABSTRACT — Background: With disturbance of the systemic blood supply, the body experiences hypoxia and stress. Under stress of any etiology, there is a non-specific rearrangement of physiological and biochemical processes. These processes occur under the influence of corticosteroid hormones. Aim: To determine the level of corticosteroid receptors in the kidneys of rats at different times after systemic ischemia-reperfusion. Methods: The study included 80 male white rats. All the animals were divided into 2 groups. A model of systemic ischemia-reperfusion was created in the main group (n=70). Further, on 1, 3, 5, 7, 14, 21 and for 35 days, we determined the level of gluco - and mineralocorticoid receptors in the kidneys. Results: In the animals of the main group, we observed a short-term period (during the first 3 days) of a decrease in the content of both glucorticoid ($p<0.05$) and mineralocorticoid ($p<0.01$) receptors. The dynamics of recovery of the level of corticosteroid receptors was 3 times faster than that of mineralocorticoid receptors. Conclusions: The dynamics of corticosteroid receptors' level in the kidneys of rats after ischemia caused by an arrest of systemic circulation show the recovery time after ischemia-reperfusion injury, which ensures the stability of an individual to hypoxia.

KEYWORDS — glucocorticoid receptors, mineralocorticoid receptors, resistance to hypoxia, ischemia-reperfusion injury.

INTRODUCTION

The complex of protective and compensatory reactions that occur under the influence of extreme hypoxia primarily involves the activation of the hypothalamic-pituitary-adrenal system. It is known that under the influence of corticosteroid hormones (CSH) under stress of any etiology, a nonspecific rearrangement of physiological and metabolic processes occurs, which is necessary to ensure the coordinated functioning of all body systems and adaptation to the pathogenic factor [1].

In case of disruption of the systemic blood supply, the effects of corticosteroid hormones can be significantly distorted due to changes in the sensitivity of target tissue receptors and disorders in the processes of hormone biotransformation, which can cause serious maladaptive consequences [2].

The kidney is an organ that is very sensitive to hemodynamic disorders and ischemia [3]. Therefore, the study of the reaction of resistance to hypoxia after ischemia-reperfusion disorders is an urgent issue.

Aim:

To determine the level of corticosteroid receptors in the kidneys of rats at different times after systemic ischemia-reperfusion.

METHODS

The study was experimental in type and included 80 mature male white rats. The study was carried out in accordance with the ethical standards for the treatment of animals adopted by the European Convention for the Protection of Vertebrate Animals for Research and Other Scientific Purposes, the Federation of European Associations for the Laboratory Animal Science and the International Council for the Laboratory Animal Science.

All animals were divided into two groups:

— main group (n=70; 10 rats for each stage of observation). In this group, we created a model of systemic ischemia-reperfusion.

— control group (n=10).

At first, we gave ether anesthesia to all the animals of both groups. Then, we simulated a 5-minute stop of systemic circulation by clamping the vascular bundle of the heart intrathoracically under ether anesthesia to the rats of the main group. Further, we carried out resuscitation measures in these animals: external heart massage and artificial lung ventilation. In the control group of rats after ether anesthesia, the circulatory arrest was not reproduced.

We evaluated the response of resistance to hypoxia after systemic ischemia-reperfusion based on

the level of gluco- and mineralocorticoid receptors in the kidney tissue homogenates. The level of receptors was determined on the analyzer "StatFox 2100" by the method of enzyme immunoassay, using standard test kits ELISA Kit (China) from Cloud-Clone Corp (USA). We studied the level of corticosteroid receptors on 1, 3, 5, 7, 14, 21 and 35 days.

Statistical processing of the material was carried out using the program "STATISTICA 7.0". The confidence of differences between quantitative indicators was evaluated using the Mann-Whitney test. The differences were considered significant at $p<0.05$.

RESULTS

In the animals of the main group, we observed a short-term period (during the first 3 days) of a decrease in the content of both glucorticoid ($p<0.05$) and mineralocorticoid ($p<0.01$) receptors (Fig.1, 2). This fact can be explained by the possible structural and functional rearrangement of physiological/biochemical systems for the implementation of a complex of adaptation reactions to the changed internal conditions. Starting from the 5th day of the post-resuscitation period, we observed a gradual increase in the activity of glucocorticoid and reduced activity of mineralocorticoid receptors. Moreover, the level of glucocorticoid receptors gradually increased, and by the end of the follow-up period was significantly higher by 23% ($p<0.05$) (Fig. 1).

The dynamics of the increase in the level of mineralocorticoid receptors were recorded 2 weeks after ischemia-reperfusion injury (Fig. 2). By the end of the follow-up period, the level of mineralocorticoid receptors did not significantly differ from those in the control group of animals.

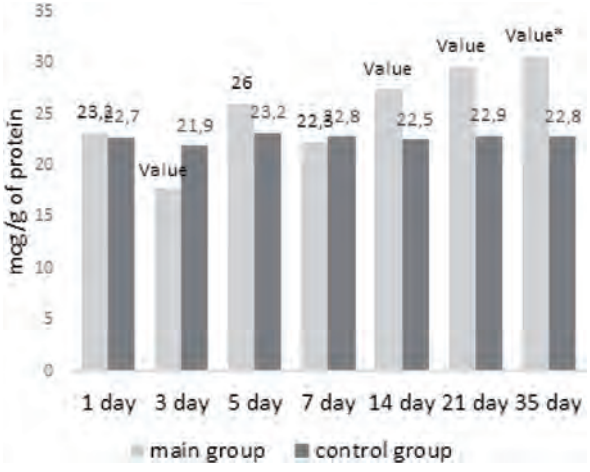

Fig. 1. *Dynamics of the content of glucocorticoid receptors in the kidneys of rats in the post-resuscitation period (*p<0.05)*

DISCUSSION

Adaptation to hypoxia is a reaction aimed at maintaining the vital activity of the body in conditions of oxygen deficiency. It is controlled by central, intercellular, and intracellular regulatory mechanisms. During the period of an urgent generalized response to hypoxia, there is a simultaneous activation of various signaling systems of regulation and an increase in the urgent resistance of the body to oxygen deficiency [4]. The leading role in this process is played by the hypothalamus—pituitary—adrenal system and its main mediators-catecholamines and corticosteroids. During this period, multiple subordinate signaling systems are also activated, which ensure the formation of an urgent compensatory protective reaction of the body in response to hypoxia [1].

The kidney is one of the main target organs for systemic circulatory disorders. Studies of various scientists have shown that aldosterone, through mineralocorticoid signaling, plays a key role in the pathogenesis of ischemia-reperfusion kidney damage, manifested in the development of progressive renal dysfunction, proteinuria, glomerulosclerosis, tubulointerstitial fibrosis, etc. [2, 3, 5]. According to Barba-Navarro R. I. blockage of mineralocorticoid receptors with spironolactone before or even after ischemia prevents acute functional and structural damage caused by ischemia-reperfusion [6].

CONCLUSION

After ischemia-reperfusion injury, a dynamic increase in the level of glucocorticoid receptors in the kidney is observed from day 5. Recovery of the level of mineralocorticoid receptors is observed from the 14th day. Consequently, in the early post-resuscitation period, the kidney is most vulnerable to the effects of remodeling factors and fibrosis.

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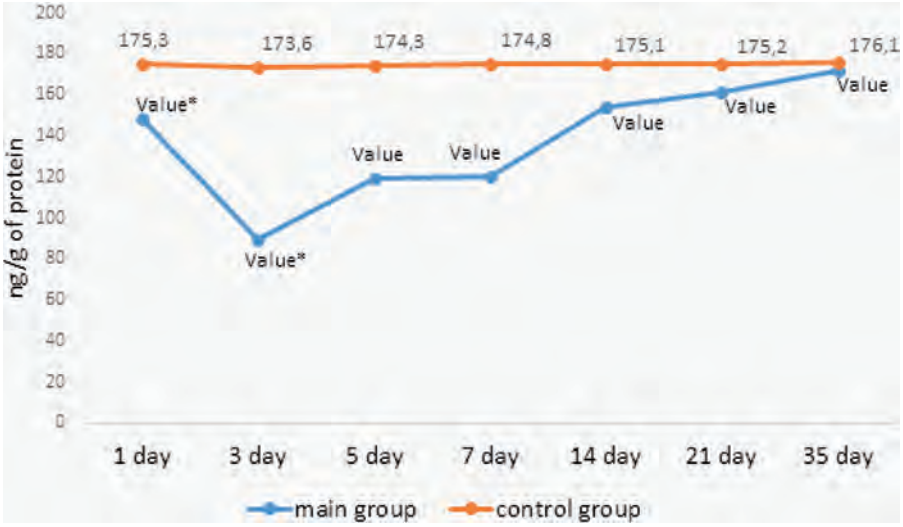

Fig. 2. *Dynamics of mineralocorticoid receptor content in rat kidneys in the post-resuscitation period*

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BACTERIAL SYMBIOSIS IN COMPLICATED ULCERS: THE PATHOGENETIC HYPOTHESIS

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ABSTRACT — Aim of this paper is to estimate crystallogenic properties of gastric mucosa in connection with its microbial contamination. We investigated crystallogenic properties of some biological substrata (gastric mucosa, gastric mucosal layer homogenates) in 12 healthy individuals and 30 patients with ulcer disease complicated in 12 cases by perforation, bleeding or penetration. Biological substrata were received at fibrogastroduodenoscopy. Estimation of crystallogenic properties of biological material was accomplished by classic crystalloscopy. Biological substrata crystalloscopic investigation was accompanied by its traditional microbiological study for Helicobacter pylori and detection of other microorganisms. Our data allow to suppose dual contamination of stomach mucosa both by Helicobacter pylori and Providencia or Morganella. This combination caused elevation of gastric mucosa crystallogenic properties that provoked formation of ulcer. Procrystallogenic potential of this symbiosis may be an important link to the pathogenesis of ulcer disease which realized through microorganism-associated mucosal damage and the progression of complications.

KEYWORDS — ulcer disease, microbe-associated crystallogenesis, Helicobacter pylori, Providencia, Morganella.

INTRODUCTION

Microogranism susceptibility to crystallization was stated long ago. So, it was shown by V.F. Chubukov (1982), that bacteria can form many variants of crystal and pseudocrystal structures [1]. These data was confirmed by other investigators [2, 3, 5, 6]. We named illustrated phenomenon as «microorganism-associated crystallogenesis» (MAC). In our opinion, it has number of functions, such as protective, pathogenic etc [2, 3, 5]. Literature data analysis has shown, that in natural conditions a protective function of MAC is dominated, and a pathogenic function realizes at bacterial antagonism or infection process [2].

Special variant of MAC is the bacterial symbiosis with a high crystallogenic activity. Example of this symbiosis is microbial induced film-formation in catheters.

Aim of this paper:
to estimate crystallogenic properties of gastric mucosa in connection with its microbial contamination.

MATERIAL AND METHODS

We investigated crystallogenic properties of some biological substrata (gastric mucosa, gastric mucosal layer homogenates) in 12 healthy individuals and 30 patients with ulcer disease, among them 12 patients with ulcer complicated by perforation, bleeding or penetration. Biological substrata were obtained using fibrogastroduodenoscopy.

Estimation of crystallogenic and initiated properties of biological material was accomplished by own methods (classic crystalloscopy and comparative teziography [4]). We used 0,9% sodium chloride solution as basic substance in teziographic test.

Biological substrata crystalloscopic investigation was accompanied by its traditional microbiological study for Helicobacter pylori (Hp) or/and other microorganisms detection.

Statistical processing of the results was performed using variation statistics algorithms using Microsoft Excel 2007 and Statistica 6.1 for Windows.

RESULTS

Investigation of own and initiated crystallogenesis of gastric mucosa in individuals without gastrointestinal pathology allows to state that in this case biological material has a low crystallogenic activity. At dehydration it mainly formed numerous amorphic structures and single small crystals. In described crystalloscopic picture of gastric mucosa Helicobacter pylori was not detected in biological substrate. So, in physiological conditions bacterial flora and gastric mucosa contain bulk mucopolysaccharides [3], have no essential crystallogenic potential and do not promote material initiation of basic substance crystallogenesis in teziographic test (Fig. 1).

On endoscopy, the evidence of gastric ulcer induced by Hp infection, crystallizability and initiation potential of gastric mucosa are meaningfully higher than in healthy people ($p<0,05$). It manifested as elevation of the portion of crystalloid structures in facia caused by crystallizability rate augmentation. However, this tendency is not accompanied by complication of structural elements (structure index saving at normal level; $p>0,05$). These changes were

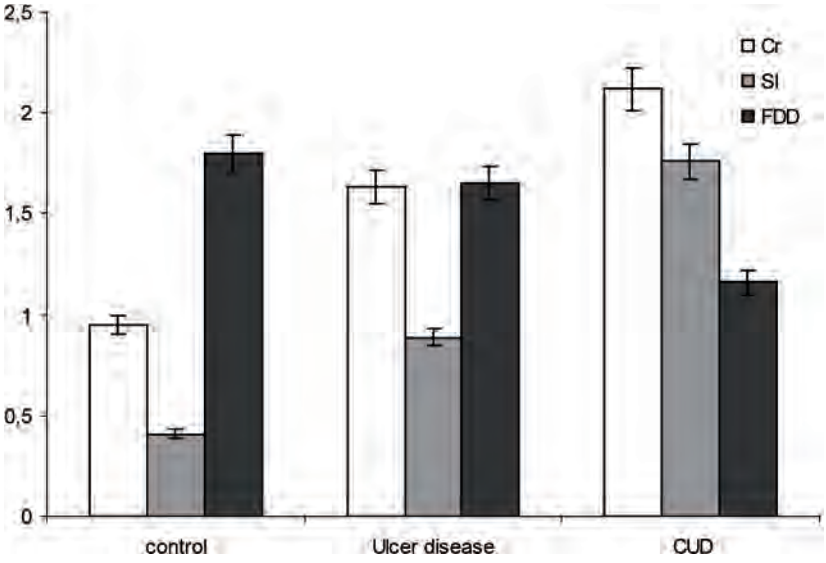

Fig. 1. *Visuometric estimation of crystallogenic properties of gastric mucosa (Cr — crystallizability, SI — structure index, FDD — facia destruction degree, CUD — complicated ulcer disease)*

corroborated by morphological and morphometric analysis of gastric mucus layers from homogenate mucosal samples. In their facias only single small crystals are presented.

At complicated variants of ulcer disease noticeable activation of crystallogenesis in investigated biological fluids is visualized. So, in this case crystallograms for homogenates of gastric mucosa and gastric mucosal layer include numerous single-crystal elements and dendrites. It caused elevation of crystallizability and structure index level in comparison with healthy people and patients with non-complicated ulcer disease rate. It is interesting, that forming crystals have rather low degree of destruction (1,42±0,31 rel. un.), which can indicate its importance in this pathogenic type of ulcer disease. This tendency is visualized in teziographic facias too. At morphological analysis in homogenates of the gastric mucosal layer, its high crystallogenic properties were discovered. Crystallograms of this biological substrate contained dendrites. It is very important, that at microbiological study in all biological materials Providencia or Morganella bacteria were marked out in addition to Hp. Based on the above consideration we assume that its symbiosis can be an initial factor of the damaged gastric mucosa.

CONCLUSION

Our data allow supposing dual contamination of gastric mucosa both by Helicobacter pylori and Providencia or Morganella. This combination caused elevation of gastric mucosa crystallogenic properties that provoked formation of ulcer. Procrystallogenic potential of this symbiosis may be an important link to ulcer development, which realized through microorganism-associated mucosa damage (result of MAC activation) and progression of the complications.

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STUDY OF POSSIBLE MECHANISMS OF ACTOPROTECTIVE ACTION OF CATEKHIN HYDRATE

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ABSTRACT — A study was carried out to study the possible mechanisms of actoprotective action of catechin hydrate administered at a dosage of 100 mg/kg. The animals were subjected to daily exhausting loads in the forced swimming test with a load of 10% of the animal's weight. After the end of the experiment, the rats were decapitated under chloral hydrate anesthesia (350 mg/kg) and the skeletal muscle was taken to obtain the supernatant. Using the ELISA method, the concentrations of nitric oxide isoforms (eNOS, iNOS, nNOS), PPAR (peroxisome proliferator-activated receptors) and JNK were estimated.
It was found that against the background of the introduction of the test substance, an increase in eNOS activity was observed by 48.7% ($p < 0.05$) relative to the group of negative control rats, as well as a decrease in iNOS and JNK — 1.9 times ($p < 0.05$). In comparison with the group receiving Metaprot®, the concentration of endothelial synthase in the group receiving catechin hydrate was 1.3 times higher ($p < 0.05$).
The experiment performed suggests that the actoprotective effect of catechin hydrate is likely to be associated with inhibition of pathways mediated by JNK, activation of PPAR receptors, and the effect of these compounds on NO isoforms.

KEYWORDS — actoprotectors, mechanism of action, rats, catechin hydrate.

INTRODUCTION

Catechin is flavan-3-ol, a flavan derivative with four phenolic hydroxyl groups, which exhibits a wide range of pharmacological activities, including: antioxidant, antihypoxic, antibacterial [1, 2]. It is also worth noting that this compound is capable of increasing physical performance against the background of exhausting physical exertion [3]. In view of its polyfunctional action, this compound can be classified as a group of actoprotectors.

Physical activity in natural conditions, and sometimes even exhausting physical activity, which athletes are often exposed to, have an effect on all systems of the body, including skeletal muscles. It is known that the symptomatology of musculoskeletal fatigue combines factors that limit the activity of skeletal muscles, first of all: a decrease in the level of blood flow in skeletal muscles and the intensity of metabolic processes in muscle tissue. In this case, the leading role in maintaining the proper level of muscle blood flow is assigned to the vascular endothelium [4]. It has been established that endothelial dysfunction developing during physical exertion is associated with a decrease in the catalytic activity of endothelial nitric oxide synthase (eNOS), one of the key endothelial enzymes that provides the physiological secretion of nitric oxide (NO). In addition, it is worth noting that optimal blood flow in skeletal muscle is influenced by compounds that affect PPARs (Peroxisome Proliferator Activated). This type of receptor belongs to the superfamil nuclear glycoproteins activated by ligand transcription factors, of synthetic or endogenous origin.

Along with oxidative stress, apoptosis is also one of the main pathogenetic mechanisms of destruction of skeletal muscle myocytes under conditions of intense muscle work, and its modulation is a new promising direction for restoring the functional activity of striated muscles. According to recent studies, c-Jun-terminal kinase (JNK) may be a potential target for regulating apoptotic processes [5].

Thus, based on the available literature data, it can be assumed that by acting on specific pathological targets, it will be possible to reduce the manifestations of muscle dysfunction.

Objective:
to study the possible mechanisms of the actoprotective action of catechin hydrate.

MATERIALS AND METHODS

The experiment was carried out in accordance with the "Guidelines for the conduct of preclinical studies of drugs, ed. A.N.Mironov (2012 ed.) [6]. The animals were kept in the vivarium of the PMFI-branch of the Volgograd State Medical University (Volgograd, Russia). The study was carried out on male Wistar rats weighing 220–230 g. All animals were divided into 4 experimental groups. During the experiment, the rats

were kept in standard vivarium conditions (natural mode of light change, temperature, relative humidity, standard diet of laboratory animals, weekly change of bedding and cages, fixed time of feeding and drinking) in compliance with the International Recommendations of the European Convention for the Protection of Vertebrate Animals used in experimental studies. The animals were subjected to daily exhausting loads in the Forced Swimming test with a load of 10% of the animal's weight [6]. Positive control (PC), the first group of animals (rats swam with days of rest), the second — negative control (NC) group of untreated animals (0.9% sodium chloride solution in an equivalent volume), the third — received the studied substance catechin hydrate at a dosage of 100 mg/kg [7], the fourth group received actoprotectorMetaprot® at a dosage of 25 mg/kg [8].

The substance catechin hydrate, as well as the reference drug, were administered per os for 10 days 60 minutes before the expected load. After the end of the experiment, the animals were decapitated under chloral hydrate anesthesia (350 mg/kg) and the skeletal muscle was taken to obtain the supernatant. In the obtained biomaterial, using the ELISA method on a microplate reader (Tecan Infinite F50, Austria), the concentrations of nitric oxide isoforms (eNOS, iNOS, nNOS), PPAR (peroxisome proliferator-activated receptors) and JNK (c-Jun-terminal kinase) were estimated. The results were processed using the STATISTICA 6.0 software.

RESULTS

During the experiment, it was found that in the group of rats without pharmacological support (NC), after prolonged depleting loads, there was a decrease in the concentration of (eNOS) — endothelial synthase relative to the PK group by 53% ($p < 0,05$), while the concentration of inducible NOS was 2,7 times higher in comparison with the same group ($p < 0,05$). Relative to the PK group, a significant increase in the concentration of PPAR and JNK was found 4,6 and 5,1 times (Fig. 1).

The course use of catechin hydrate led to an increase in the concentration of eNOS — by 48,7% ($p < 0,05$) and a decrease in iNOS, as well as JNK — by 1,9 times ($p < 0,05$) (Fig. 2). However, it should be noted that the concentration of endothelial NOS was 1,3 times higher than that of the group receiving Metaprot® at a dosage of 25 mg/kg ($p < 0,05$). The PPAR concentration index did not differ statistically significantly from the group of NC rats.

The introduction of Metaprot® promoted an increase in PPAR concentration by 2,7 times ($p < 0,05$) and a decrease in JNK by 1,7 times ($p < 0,05$) in relation to the group of NK animals. Against the background of administration of the drug Metaprot®, there was no significant effect on the content of nitrogen monoxide isoenzymes in comparison with the group of NK rats.

It should be noted that neither the use of catechin hydrate nor Metaprot® led to a significant change in the expression of the isoenzyme of neuronal synthase (nNOS).

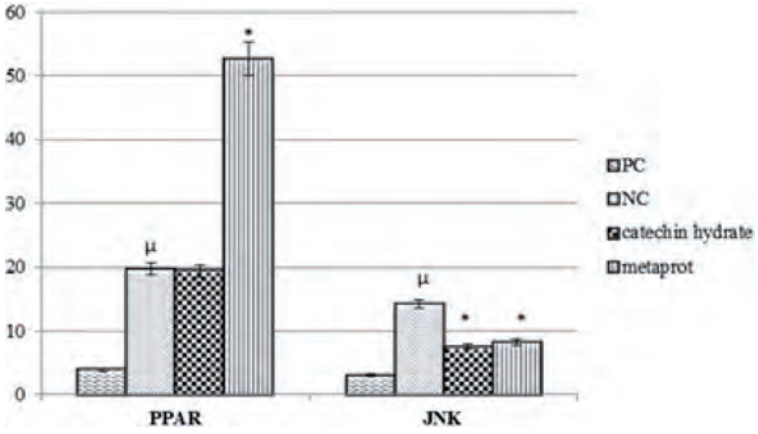

Fig. 1. *Changes in the concentration of PPAR and JNK against the background of the introduction of the studied substance catechin hydrate in the supernatant of the skeletal muscle of rats exposed to exhausting physical exertion*

Note: *μ — statistically significant relative to the group of animals PK (Student's t-test, $p < 0,05$); * — statistically significant relative to the group of NC animals (Student's t-test, $p < 0,05$)*

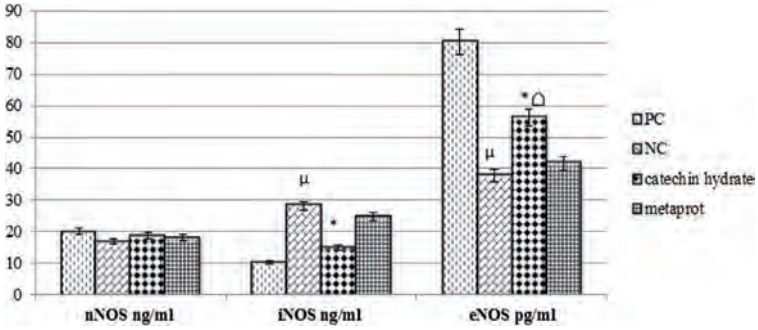

Fig. 2. *Changes in the concentration of NOS isoforms against the background of the introduction of the studied substance catechin hydrate in the supernatant of the skeletal muscle of rats subjected to exhausting physical exertion*

Note: *μ — statistically significant relative to the group of animals PK (Student's t-test, $p < 0.05$); * — statistically significant relative to the group of NC animals (Student's t-test, $p < 0.05$); ⌂ — statistically significant relative to the group receiving Metaprot®*

CONCLUSION

The use of catechin hydrate increased the catalytic activity of eNOS by 48,7% (p <0,05) and 1,3 times (p <0,05), relative to the group of NK rats and the group receiving Metaprot, respectively. Against the background of oral intake of the test substance, a decrease in the concentration of JNK of 64,3% (p <0,05) was noted relative to the group of NK animals. As a result of the experiment, it can be assumed that the actoprotective effect of catechin hydrate may possibly be associated with inhibition of pathways mediated by JNK, activation of PPAR receptors, and the effect of these compounds on NO isoforms.

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PHARMACOKINETICS AND PHARMACODYNAMICS OF CHAGA BIRCH MUSHROOM COMPONENTS (INONOTUS OBLIQUUS)

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ABSTRACT — The study presents an analysis of the available scientific literature and outcomes of clinical trials on biologically active components of the birch Chaga mushroom *Inonotus obliquus* (*I. obliquus*). Our experimental and clinical studies were aimed at testing a novel plant-based substance for patients with cancer or tumors of various locations. The paper investigates pathogenetic mechanisms and effectiveness of chaga mushroom extracts depending on the topography of the tumor, the stage of the disease and the age of patients, as well as the questions of pharmacokinetics and pharmacodynamics of the components of *I. obliquus*.
As a result of the analysis of the obtained data, the pathogenetically justified clinical application of extracts of the birch chaga mushroom *I. obliquus* in patients with oncological pathology was conceptually presented.
The study allowed us to reveal possible mechanisms of pathogenetic effects of the birch chaga mushroom (*Inonotus obliquus*) extracts obtained by different extraction methods. Analysis of the available data enabled us to deepen our understanding of the effectiveness and mechanisms of the effect of the *I. obliquus* extracts on tumors of various localization. Finally we present recommendations on predicting the possibility of using the *I. obliquus* to improve outcomes in patients with cancer of various etiology and locations.

KEYWORDS — chaga mushroom, Inonotus obliquus, inotodiol, glycans, neuroprotector, hyperglycemia, antioxidants, stroke, apoptosis, proliferation, regeneration, malignancy, cancer.

RELEVANCE

At the current stage of development of medicine it is of particular importance to search for substances that can be used in prevention and supporting measures during treatment of cancer and neurodegenerative diseases. In contrast to strong synthetic drugs with numerous negative side effects which sometimes exceed their benefit, medicinal plants can be assumed to be effective and safe. The analysis by Yen-Nien Hou, Gary Deng, Jun J. Mao (2020) of the data from the Integrative Medicine Service at Memorial Sloan Kettering Nassau cancer center (USA) allowed us to establish that chaga mushroom is in the first place out of the 10 most popular herbs/supplements in 2018 [11, 33]. The chaga mushroom, *Inonotus obliquus* (*I. obliquus*), has been a well-known traditional medicine remedy since the time of Avicenna [1, 3]. However, the systematic pharmacological study of the active substances of *I. obliquus* was started only in the middle of the 20th century, when fundamental medicine claimed the medicinal advantages of the fungus *Inonotus obliquus* in experimental studies with the suppression of cancer cell growth [26]. The chemical composition and spectrum of the therapeutic use of *Inonotus obliquus* as raw material, as well as methods for assessing the resource potential of this fungus, are presented in numerous studies despite the background of insufficient data on the molecular mechanisms of action of mushroom extracts [17]. At the present stage, the medicinal properties of *I. obliquus* have been studied only by 10% [28]. At the same time, the available scientific literature on this issue considers to a greater extent the results of experiments performed on mice, mongrel and genetically modified, or on Danio fish, which is a key factor for choosing the direction of research in the study and systematization of data on the mechanisms of their action on the human body.

Over the past fifty years, ideas about the use of chaga extracts as additives to essential medicines are based mainly on the results of preclinical studies, with a small number of adequately planned experiments and a limited number of studies conducted on cancer patients with cancer of various topography and etiology [4]. Therefore, despite numerous experimental and biochemical studies, the available data on the biological effects of individual components at the present stage does not allow us to give an exhaustive pathogenetic justification for the use of popular additives from chaga mushroom, registered and obtained by various methods [24]. There is also insufficient information to fully decipher the mechanisms of action, substantiate the effectiveness of drugs from the chaga mushroom, prove their advantages and minimize the risks

of side effects. This analysis of available data is also not presented in the archive database of the Integrative Medicine Service at Memorial Sloan Kettering cancer center, which provides a summary of research results indicating the intended uses, side effects, and interactions of herbal medicines for approximately 284 dietary supplements [10].

The known antitumor effect of glycans and glucans that are part of *I. obliquus* is mainly associated with the induction of increased immune activity, and their effect in the composition of chaga extract on malignant tissue has not been sufficiently studied, there are only a few experiments in Vitro confirming their inhibitory effect on the proliferation of cancer cells [5].

Critical unsolved problems in the use of chaga mushroom (*I. obliquus*) extracts and the lack of systematic analysis of available materials on this issue require scientific research to be directed on these matters.

The aim of the study was to generalize data on the medicinal properties of the birch chaga mushroom (*I. obliquus*) and conduct a comparative analysis of the results of its usage in tumors of various locations and etiology.

In this regard, the following tasks were solved:

1. To provide an analysis of available literature data about the current state of the studied issue.
2. To obtain a comparative description of the results of treatment of cancer patients with tumors of different localization and etiology.
3. To determine the possible mechanisms of pathogenetic effects of extracts of the birch chaga mushroom (*Inonotus obliquus*) obtained by different extraction methods based on the available data.
4. To evaluate the effectiveness of the extracts of *Inonotus obliquus* on tumors of various localization.
5. Conceptually substantiate the clinical use of *Inonotus obliquus* extracts in patients with cancer.

MATERIALS AND METHODS

We reviewed available literature data on clinical use of extracts of the *Inonotus obliquus* for the last 20 years, as well as available archival and scientific materials on the results of treatment of patients with cancer of various etiologies and topography of maligning structures.

RESEARCH RESULTS

In contrast to the studies by Draggendorf G.L. (1864) [7], which did not discover useful substances in the birch chaga mushroom (*Inonotus obliquus*) and the presence of alkaloids and glycosides in its content, many researchers found more than 10 amino acids, 40% of which are glutamic acid, tyrosine, serine, threonine, leucine, methionine, histidine and lysine, as well as polysaccharides, in addition to numerous other chemical elements [6, 19]. Previously, data on the health properties of fungal polysaccharides were obtained by analyzing the chemical composition of 651 species and 7 infraspecific taxa from 182 genera of higher homo- and heterobasidiomycetes. It was found that, despite the different chemical composition, the majority of fungal polysaccharides belong to the group of beta-glucans, they have beta (1-->3) bonds in the main chain of glucan and additional beta (1-->>6) branching points that cause their antitumor effect. Most of the clinical evidence for antitumor activity comes from the polysaccharides like lentinan, PSK (krestin), and schizophyllan. It was found that the effectiveness of high-molecular-weight glucans overwhelms than that of low-molecular-weight glucans.

Satoru Arata, Jun Watanabe, and Masako Maeda (2016) studied the actual effect and underlying mechanisms of the effect of continuous intake of an aqueous extract from *I. obliquus* on the suppression of Lewis lung carcinoma growth and spontaneous metastasis in mouse models [2]. After 3 weeks of continuous intake of the extract at a dose of 6 mg/kg/day, the authors obtained significant suppressive effects: in models of tumor-bearing mice, a 60% reduction in the tumor was observed, while in metastatic mice, the number of maligning nodules decreased by 25% compared to the control group. In addition, mice treated with *I. obliquus* extract showed not only tumor reduction, but also inhibition of vascularization.

Eid J.I., Das B. (2020) consider *Inonotus obliquus* to be one of the most powerful antioxidants in the world [8]. The authors note that the therapeutic effects of chaga components are well characterized only in vitro; the effects of development in vivo are not described in detail. Using the example of Danio fish, the authors identified the influence of *I. obliquus* on the cell cycle and apoptosis. Polysaccharides of chaga mushrooms, in addition to phenolic compounds, included xylulose, rhamnose, mannose, glucose, inositol, and galactose. Staining with the DNA-binding dye acridine orange showed that polysaccharides of *Inonotus obliquus* alleviate oxidative stress [8]. Flow cytometric analysis using H2DCFDA, which specifically binds to fragmented DNA in cells, showed significantly reduced levels of intracellular reactive oxygen species (ROS) ($p < 0.05$), which in turn reduced apoptosis in developing embryos. Analysis of the cell cycle by measuring the DNA content using flow cytometry showed that chaga polysaccharides moderately delay cells at the G1 stage, thereby inhibiting cell proliferation, which can be further studied in cancer research. In general, temporary

exposure to chaga polysaccharide extract reduced the volume of intracellular reactive oxygen species (ROS) and contributed to the normal development of Danio fish.

Li Z., Mei J., Jiang L., Geng C., Li Q., Yao X., Cao J. (2019) found that tacrine, the first drug licensed for the treatment of Alzheimer's disease, induces apoptosis in HepG2 cells by generating reactive oxygen species (ROS) and mitochondrial dysfunction [18]. The authors studied the possible protective effect of polysaccharides from *I. obliquus*, obtaining evidence that *I. obliquus* polysaccharides reduce tacrine-induced apoptosis in HepG2 cells. The experiment also observed inhibition of tacrine-induced ROS generation, 8-OHdG formation in mitochondrial DNA, and loss of mitochondrial transmembrane potential when using *I. obliquus* polysaccharides. In addition, the intake of *I. obliquus* extract reduced the release of cytochrome C and activation of caspase-3 induced by tacrine. These data suggest that fungal polysaccharides may inhibit tacrine-induced apoptosis in HepG2 cells. This protection is mediated by an antioxidant cytoprotective mechanism [18]. Similar hepatoprotective properties of *I. obliquus* extract were proved by studies of Hong K.B., Noh D.O., Park Y., Suh H.J. (2015) [10].

Nguyen T.M.N., Le H.S., Le B.V. et al. (2020) note that the lanostane triterpenoid, inotodiol, is found exclusively in the chaga mushroom; the authors found evidence that purified inotodiol has activity to suppress mast cell function in vivo [23]. Inotodiol also relieved symptoms similar to those observed in the ovalbumin-induced (cOVA) mouse food allergy model. As with untreated 70% ethanol extract of chaga mushroom (320 mg/kg), oral administration of inotodiol (20 mg/kg), both for prevention and treatment, was accompanied by a significant reduction in the manifestations of allergic symptoms and inflammatory lesions in the small intestine that appear after repeated oral administration of cOVA. Despite the fact that inotodiol (20 mg/kg) and chaga mushroom extract (320 mg/kg) acted to the same extent, it was found that the immunological mechanisms underlying these effects differ from each other. The authors illustrated the evidence obtained from several in vivo analyses, including experiments with passive systemic anaphylaxis mediated by mast cells, activation and proliferation of adaptively transferred antigen-specific T cells, and production of IgG1, IgE, and IgA immunoglobulins by antigen-specific B cells. Inotodiol selectively inhibited mast cell function without significantly affecting other immune responses, while crude chaga mushroom extract randomly suppressed a variety of immune responses. This indicates the presence of several immunomodulating biologically active components in the chaga mushroom that act on various targets. The strong anti-allergic activity of inotodiol, as well as its remarkable selectivity against mast cells, makes it an excellent tool of choice for the treatment of food allergies with high efficiency and no side effects.

Many authors have described the positive antitumor effect of *I. Obliquus* and its extracts containing the trioterpenoid inotodiol. Nikitina S.A., Khabibrakhmanova V.R., Sysoeva M.A. (2016) summarized data on the chemical composition of triterpene and steroid compounds isolated from chaga mushroom grown in natural conditions or in synthetic culture [25]. Particular attention was paid to the biological activity of chaga mushroom extracts and these specific compounds against various cancer cell lines in vitro and in vivo. This analysis demonstrated some common features in the inhibition of growth of various cell lines by components of the chaga fungus. In this context, triterpene compounds containing an OH group at C22 and a side chain unsaturated bond are the most active [25]. Zhang P., Cao X., Li C., et al. (2016) studied the role of the squalene synthase gene isolated from *Inonotus obliquus* in triterpene synthesis by detecting squalene in vitro in a reaction mixture of particles using high-performance liquid chromatographic analysis [34]. The positive effect of treatment with the use of triterpenoid inotodiol have been demonstrated in hepatoma, leukemia, colon cancer and cervical cancer. Anti-carcinogenic effects of cell growth inhibition in lung carcinoma have also been shown. Due to the identified oncostatic activity of *I. obliquus*, Sun Y., Yin T., Chen X. H., Zhang G., et al. (2011) suggested using polysaccharide-triterpenoid complexes of *I. Obliquus* as components of heterogeneous inhibitors of cancer cell proliferation [28]. Zhang S.D., Yu L., Wang P. Kou, Li J. et al. (2019) studied the potential mechanisms of inotodiol for HeLa cell migration, invasion and apoptosis via the p53-dependent pathway, transwell invasion, flow cytometry, caspase-3 activity analysis and Western blot analysis, as well as the participation of the p53 signaling pathway in anti-metastatic and Pro-apoptosis [35]. In addition, the function of the p53 tumor suppressor was further verified by small interfering RNA. The dependence of the effect of inotodiol on its concentration was established. The cytotoxic effect on HeLa cells at concentrations above 25 microns significantly inhibits the growth of HeLa cells and even induces apoptosis. This result was further confirmed by analysis of cell proliferation and morphology. Analysis of in vitro wound healing and transwell invasion showed that treatment with inotodiol in low concentrations significantly inhibits cell migration and invasion in a dose-dependent manner.

Reduced levels of matrix metallopeptidase-2 (MMP2) and matrix metallopeptidase-9 (MMP9) also depend on the drug concentration. Inotodiol significantly induces apoptosis of tumor cells through its effect on annexin-V-FITC, which is associated with activation of pro-apoptotic PARP proteins, partition of caspase-3 and Bax expression, and inhibition of the anti-apoptotic Bcl-2 protein expression. The antitumor activity of inotodiol can be inhibited by switching off the tumor suppressor p53. Pretreatment of p53-specific small interfering RNA (si-p53) significantly inhibits inotodiol-induced apoptosis of HeLa cells and reduces the activity of caspase-3. Moreover, the inhibitory effect of inotodiol on tumor migration and invasion was blocked by p53 knockdown.

Zhang X., Bao C., Zhang J. (2018) investigated the effect of inotodiol (lanostane triterpenoid) on the cellular growth of breast tumors in diabetic conditions [36]. The research was conducted on a model of breast cancer against the background of diabetes in female Sprague-Dowley rats, caused by the administration of streptozotocin (STZ) at a dose of 35 mg/kg, followed by the induction of breast cancer 7,12-dimethylbenzanthracene (DMBA) at a dose of 10 mg/kg. Histological examination of the pancreas and of breast tumor proliferation was conducted by immunohistochemical methods with PCNA staining, Tunel method for apoptosis. The results of the study showed that inotodiol reduces blood glucose levels in SD rats, as well as reduces the level of cholesterol, triglycerides and high-density lipoproteins in blood plasma. Expression of the PCNA proliferation marker was reduced when taking inotodiol. It lowered the expression of β-catenin and its downstream targets (c-Myc and cyclin D1) with subsequent induction of apoptosis. Evidence-based results indicate that inotodiol regulates blood glucose levels in diabetic rats, participates in regulating of proliferation while inhibiting breast tumor progression, inducing apoptosis through reduced regulation of β-catenin signaling [36].

Anti-carcinogenic effect with targeted effects of *I. obliquus* extracts are shown against several cancer cell lines in the absence of cytotoxic effects to normal cells. The optimal concentration of these complexes was estimated at 150 mg/ml. Results of oral doses of *I. obliquus* polysaccharide complexes showed suppression of melanoma cell growth. Antihyperglycemic and antioxidant effects were demonstrated in experiments on mice with alloxan diabetes; data were obtained on lowering blood cholesterol levels and pancreatic regeneration. A number of studies have demonstrated the antimicrobial activity of unknown components of *I. obliquus*, which also have a clear bactericidal effect on a number of strains of Mycobacterium smegmatis and Francisella tularensis. Based on these properties, dental films based on nano-particles of chaga have been developed for the treatment of inflammatory bacterial diseases of the oral mucosa.

Chaga mushroom glycans are considered pathogenic associated molecular patterns (PAMP), similar to molecules that bind to specific pattern recognition receptors (PRRs) on the surface of immune cells. This interaction induces activation of a signaling cascade that directs the synthesis of a specific repertory of immune effector molecules [30]. Wasser S.P. (2011, 2014, 2017) noted the importance of studying these natural sources as a means of treating cancer [31]. Many fungal biopolymers have immunotherapeutic properties, contributing to the inhibition of growth and inducing the destruction of tumor cells. Although the mechanism of their antitumor action is still not fully understood, the development of methods for stimulating and modulating key immune responses with fungal polysaccharides is a central task in medicine in general and in oncology in particular [12]. Some of the fungal polysaccharides have passed through phase I, II, and III clinical trials and are widely and successfully used in Asia for the treatment of various types of cancer and other diseases [26]. Based on the chemical composition, it is assumed that the components of the chaga mushroom have 126 therapeutic functions, including antitumor, immunomodulatory, antioxidant, antiischemic, cardiovascular, antihypercholesterolemic, antiviral, antibacterial, antiparasitic, antifungal, detoxifying, hepatoprotective and antidiabetic effects [32]. Given that the mechanism of their antitumor action has not yet been fully studied, the issues of stimulation and modulation of immune responses by chemical components of chaga are of key relevance at the present stage [29].

The results of more than 600 clinical studies testing the effects of fungi on humans have been published in numerous papers [21]. Mushrooms are an extensive and at the same time almost inexhaustible source of powerful new pharmaceutical products [25]. In particular, and this is the most important thing for modern medicine, they are an unlimited source of polysaccharides [4]. These polysaccharides have different chemical compositions, and most of them belong to the group of beta-glucans; they have beta (1-->3) bonds in the main chain of glucan and additional beta (1-->>6) branching points that provide mechanisms for their antitumor action. It appears that high-molecular-weight glucans are more effective than low-molecular-weight glucans [6]. The same opinion is shared by Xin X., Qu J., Veeraraghavan V.P., (2019), who showed existing achievements in research on the mechanisms of isolated fungal polysaccharides,

in particular (1-->3) -β-D-glucans, which can cause various cellular responses, such as the expression of cytokines and nitric oxide, which carry out antitumor mechanisms indirectly through stimulating T cells or binding other molecules of cellular immunity, such as polypeptides and proteins, conjugation of which always has a strong antitumor activity [32].

Cytotoxic bioactivity of *I. obliquus* was established in four human lung adenocarcinoma cell lines with different p53 status (A549, H1264, H1299, and Calu-6) [22]. According to the authors, the analysis of apoptosis induction tests with MeOH *I. obliquus* extract showed a decrease in cell viability in all lung cancer cell lines, and the cytotoxicity of these compounds was mediated by apoptosis through activation of caspase-3. Directed increase in bioactivity through fractionation of MeOH extract and chemical study of its cytotoxic hexano-soluble and Ch2Cl2-soluble fractions led to the isolation of eight triterpenoids, including a new lanostane-type triterpenoid called chagabusone A. The structures of the isolates were elucidated by high-resolution spectroscopic analysis. Among the isolated compounds, some of them showed the most powerful cytotoxic activity in all studied human lung cancer cell lines, with IC50 values varying from 75.1 to 227.4 microns.

In the treatment of cancer, fungi can inhibit metastasis due to the presence of arctigenin, lignan, in its composition—an important component and a representative of the group of phenolic compounds of plant origin. As is known, the basis of tissue malignancy inhibitors, cytostatics, contains organic compounds of the aromatic series, in whose molecules the hydroxyl groups OH– are bound to the carbon atoms of the aromatic ring. Arctigenin refers to phytoestrogens that are metabolized in the same way as estrogen hormones. The use is accompanied by a reduction in the risk of breast cancer after menopause, which is due to the fact that lignans have a milder effect on cells than human estrogen, effectively blocking the action of powerful endogenous human estrogen [27]. At the same time, the probability of malignancy in the mammary gland, which depends on growth hormones, is reduced by inhibiting individual proteins (NPAT proteins) necessary for the formation of cancer cells, paralyzing the ability of maligning cells to reproduce. Studies have shown that arctigenin is effective in the treatment of lung, liver, and stomach cancer [19].

Kang J.H., Jang J.E., Mishra S.K., et al. (2015) studied the effect of various fractions and components of the chaga mushroom (*Inonotus Obliquus*) on viability and apoptosis in human colorectal cancer (CRC) cell lines, and revealed the most effective inhibition of tumor growth by a single component identified by pulsed nuclear magnetic resonance (NMR) as ergosterol peroxide with known antiproliferative and apoptotic activity [13]. Lee H.S., Kim E.J., Kim S.H. (2015) cultured human colon cancer HT-29 cells in the presence of 2.5–10 micrograms/ml of *I. obliquus* ethanol extract (IOEE) and analyzed cell cycle arrest by flow cytometry and Western blot protein expression control. It was found that the treatment of cells with 2.5–10 micrograms/ml of IOEE reduces the number of viable HT-29 cells and DNA synthesis, increases the percentage of cells in the G1 phase, reduces the expression of CDK2, CDK4 and cyclin D1 proteins, increases the expression of p21, p27 and p53, and inhibits phosphorylation of Rb and E2F1 expression. Among *I. obliquus* fractions, fraction 2 (fractionated with dichloromethane) showed the same effect as EEIO treatment on cell proliferation and the level of protein associated with the cell cycle [16].

Currently, *I. obliquus* is considered a non-specific medicine for gastritis, gastric ulcer, polyposis and is used for precancerous therapy in liquid or tablet form, as a complex drug "Befungin" represented by a concentrated extract of chaga mushrooms. To establish the pharmacological properties of *I. obliquus*, studies were conducted showing the anti-ulcer activity of ethanol extract of chaga. The anti-ulcer activity of *I. obliquus* was determined in rats with gastric ulcer (ethanol-induced ulcer). Ethanol extract of *I. obliquus* (200 mg/kg) did not cause any signs of toxicity or sensitivity in rats when administered orally. Oral administration of ethanol extract of *I. obliquus* showed anti-ulcer activity in all models used. The ethanol extract of *I. obliquus* showed effective anti-ulcer activity, which could be due to the presence of various biologically active compounds.

Na H.G, Park Y., Kim M.A., et al. (2019) noted that the use of chaga has numerous health benefits in alternative medicine in the treatment of obesity. Their proposed powder mixture of Chaga mushroom extracts was fermented using Lactobacillus acidophilus KCTC3925 (FCC), with the determination of an anti-cholesterol effect in a high-fat diet (HFD) in mice [20]. Analysis of the experimental results showed that the level of serum GT, GPT, and leptin, as well as the expression of hepatic COX-2 mRNA, as well as splenic COX-2 and IL-4 mRNA, were significantly higher in the HFD groups than in the control group ($P > 0.05$). With the exception of splenic IL-4 levels, these increases were significantly reduced by the addition of FCC. Expression of ICAM-1, a marker of aortic inflammation, was significantly increased in the HFD group. FCC suppressed weight gain and epididymal fat appendages, as well as inflammatory responses in the liver and spleen of HFD-fed mice.

Kazumi Sagayama, Naonobu Tanaka, Takatoshi Fukumoto, Yoshiki Kashiwada (2019) identified, after studying the effect of *Inonotus obliquus* on the proliferation of human papillary follicle cells (Hfdpc), five lanostane-type triterpenes (1-5) using spectroscopic data, of which lanosterol (1), inotodiol (3), lanost-8,24-diene-3β, 21-diol (4) and trametenolic acid (5) showed a more potent proproliferative effect on Hfpdc than minoxidil, an anti-alopecia agent used as a positive control. Lanostane triterpenes (1, 3, 4, and 5) were potential candidates for new products that can be used for hair care with a stimulating effect on hair growth [27].

Devi K.S. and Maiti T.K. (2016), in a review of patents for the extraction of biologically active substances (BAS) from chaga mushroom, showed that for decades the use of components from mushrooms was considered in the framework of ethnic medicine and was widely used in the treatment of various serious diseases [6]. Methods of extraction of pharmacologically significant fungal glucans affect their immunostimulating properties, structure, composition, solubility in water and conformation in solution. In addition, modifications of glucans depending on the particle size also contribute to a significant increase in their activity [15].

In order to increase the antitumor activity of polysaccharides and improve their clinical properties, which depend mainly on their solubility in water, the main procedures used to optimize the isolation of polysaccharides are Smith decomposition (redox hydrolysis), formolysis, and carboxymethylation [31].

Gil Y.G., Kang S., Chae A. et al (2018) developed and synthesized anisotropic porous palladium nanoparticles with full-wavelength absorption in the ultraviolet–visible–near-infrared range using concentration-dependent reduction synthesis to maximize the pharmacological activity of chaga mushroom (*Inonotus obliquus*) extract [9]. Porous Pd nanoparticles with chaga extract showed a surface antitumor effect, controlled delivery of doxorubicin via electrostatic interaction, and photothermal conversion effect under 808 Nm laser irradiation. The combined use of three approaches to cancer treatment has clearly demonstrated the feasibility of synergistic trimodal therapy. A modern platform using Pd, which is a key component of nanocatalysts, while not often used in biological applications, involves numerous applications using PD nanostructures, as well as the potential for development of new cancer treatments.

Kutaiba Ibrahin Alzand, Sabri Ünal, Mansor S. Mostafa Boufaris (2018) isolated three new lanostane-type triterpenes, a new abietane-type diterpenes, and 10 known compounds (5-14) from sclerotia of *Inonotus obliquus* [1]. Their structures were established using a combination of spectrometric methods, including infrared, 1-dimensional and 2-dimensional nuclear magnetic resonance and high-resolution electrospray mass spectrometry. During in vitro studies, the compounds showed hepatoprotective effects against D-galactosamine-induced damage in WB-F344 cells with inhibitory effects ranging from 35.4% to 83.8%. Some of the compounds showed selective cytotoxicity against Bel-7402, A549 or KB cell lines and inhibitory action against protein tyrosine kinases with half-maximum inhibitory concentrations of 23.8 and 7.4 mmol/l, respectively.

A simpler way to prepare the extract is to extract it from crushed fruit bodies of the fungus by boiling them in water (0.5 l) for 30 minutes. The yield of the extract from mushrooms will be 30% on average.

In the same volume of ethanol, an alcohol extract is prepared from the crushed fruit bodies of the fungus *I. obliquus* (0.5 l) for 5 days. The yield of the extract from mushrooms is 60% on average. The absence of side effects of the use of *I. obliquus* ethanol extract (200 mg/kg) and of signs of toxicity or sensitivity in rats after oral administration of *I. obliquus* ethanol extract with anti-ulcer activity in all models used is due to the presence of various biologically active compounds [14].

CONCLUSION

Water and alcohol extracts of *I. obliquus* that inhibit the proliferative activity of malignizing cells suggest that these fungi can potentially be used as an easily accessible source of natural antioxidants and inhibitors of the process of carcinogenesis and metastasis with promising potential in the prevention of metabolic disorders associated with reactive oxygen species (ROS). *I. obliquus* should be considered as a source for the manufacture of drugs with antioxidant, antimicrobial and antihyperglycemic activity. The most important component of chaga is betulinic acid, which ranks first in the ORAC for the level of antioxidants in food. The expressed activity of iron recovery dictates the study of the antiviral properties of the fungus *I. obliquus*, which is especially important at the present stage for the upcoming solution of issues with therapeutic and anti-epidemic measures against COVID-19. The results are encouraging, they explain and confirm the possibility of nutritional use of mushroom fruit bodies for the prevention and treatment of oxidative damage in neurodegenerative diseases, oncological pathology of tumors of various genesis and localization, as well as diseases of the pancreas and liver as hepatoprotective agents, and also as anti-allergic agents with a mild sedative effect. Chaga mushroom extracts can prevent

the development of breast, ovarian, cervical, prostate, lung, stomach, spleen, brain, and thymus cancer in the initial stages, as well as leukemia, lymphoma, and melanoma.

The effectiveness of the drugs made from extracts of the birch chaga mushroom (*Inonotus obliquus*) on tumors of various localization is high and allows us to conceptually justify the clinical application for patients with cancer. Glucan and triterpenoid components of the fungus allow for the use of *I. Obliquus* in some cases as a direct antitumor agent and a regulator of apoptosis. The use of polysaccharides from *I. obliquus* can actually prevent tacrine-induced hepatotoxicity. Analysis of the results of numerous studies provides experimental evidence confirming the prospects for the use of *I. obliquus* in the treatment of lung cancer, and reveals the molecular basis underlying its cytotoxic activity against human cancer cells. Inotodiol obtained from *I. obliquus* can be used for the prevention and treatment of not only chronic conditions such as diabetes but also breast cancer.

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BIOCHEMICAL STUDY OF MENSTRUAL BLOOD AND A MATHEMATICAL MODEL FOR DIAGNOSIS OF UTERINE MYOMAS

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ABSTRACT — Uterine myomas or fibroids (UF) are the most common tumors in women, which can cause serious complications, often require surgical interference and cause infertility. The authors proposed a comprehensive approach to solving this issue, which includes identifying the characteristic structures of the solid phase of menstrual discharge and the introduction of a mathematical model for predicting the growth rates of UF. Patients were divided into three groups of women of reproductive age. The control group included 41 relatively healthy patients. The second group consisted of 40 women with simple UF (group 1), and 30 women with proliferating UF were included in the third group (group 2). Development and implementation of a method for diagnosing UF, which includes the study of morphological structures of solid phase of menstrual blood and levels of ligands inducing apoptosis and proliferation, allows to find the most optimal solution for this clinical task. A mathematical model is proposed for predicting the growth rates of UF. The safety and atraumatic method of morphological analysis of menstrual fluids makes it possible to apply it effectively in the work of an outpatient clinic.

KEYWORDS — uterine fibroids, proliferating uterine fibroids, proliferation, apoptosis, menstrual blood.

INTRODUCTION

Uterine myomas or fibroids (UF) are the most common benign tumors of female genital organs in the world [1, 2, 3, 4], affecting at least 20–40% of women during their lifetime. Moreover, it is UF that accounts for one third to half of all hysterectomies. According to the results of a study carried out by a group of scientists headed by I.S. Sidorova in 2000, it was proposed to distinguish two clinical and morphological types of the disease: simple and proliferating UF [3]. Moreover, at the same time, other researchers studied this aspect and came to the similar conclusions [1]. Simple and proliferating UF can vary on a number of features, such as, the location of nodes, their number, size, ultrasound characteristics.

At the cellular level, simple UF is characterized by low activity of proliferation and angiogenesis processes in combination with increased synthetic activity and severity of the stromal component [4]. At the same time, proliferating UF is characterized by rapid growth and high mitotic activity [5]. Biological fluids, such as menstrual blood, cervical mucus and endometrial lavage, contain markers that allow one to suspect and identify the presence of abnormalities in the organs of the reproductive system [1, 6, 7, 8, 10].

The development and implementation of a method for the diagnosis of UF, including the study of the morphological structures of the solid phase of menstrual blood and the levels of ligands inducing apoptosis (LIA) and proliferation (LIP), makes it possible to find the most optimal solution to this clinical problem [9, 11].

The aim of the study was to develop a diagnostic method for proliferating uterine fibroids by determining the structural features of menstrual blood, identifying LIA and LIP in them, and to make a mathematical model for predicting the possible growth rate of UF.

MATERIAL AND METHODS

Women of reproductive age (23–45 years old) were divided into three groups. The investigation was held at the Gynecology and Obstetrics Department of Astrakhan State Medical University. The control group included 41 relatively healthy women. The second group consisted of 40 women with simple UF (group 1), and the third group consisted of 30 women with proliferating UF (group 2). The investigation was held in accordance with the universal ethical standards.

The distribution of women into groups with simple and proliferating UF, as well as the exclusion of women with «false» rapid growth was carried out on the basis of the results of histological examination of myoma nodes, ultrasound data, acute phase proteins, clinical picture and growth rates of myoma. The study included only patients with a «true» type of rapid UF growth. In the course of the work, data on the number, localization, and size of myoma nodes were analyzed, obtained by ultrasound performed on days 5–7 of the menstrual cycle.

Menstrual blood was taken in the most abundant days of menstruation. The obtained samples were centrifuged at 3000 rpm for 5 min. The resulting supernatants were used for further morphological and immunochemical studies. The supernatant was dropped onto a glass slide and covered with a coverslip. The material obtained was dried at room conditions for 2–3 days [12].

The analysis of the structures of the solid phase of menstrual fluid formed during the edge dehydration was carried out using microscope Leica MZ 12.5 (Switzerland) equipped with a Leica ICC 50 digital camera.

The content of LIP and LIA in the menstrual blood supernatant was determined by enzyme immunoassay using Bender MedSystems kits (Austria) [11].

The study results were processed using Microsoft Office 2010, Statistica software (StatSoft Inc., USA version 8.0). To study and compare the data obtained, the Kruskal-Wallis, Kolmogorov-Smirnov tests, as well as the method of correlation analysis were used. Description of qualitative features was reproduced in percent (%), absolute values (n/N) and contingency tables with the definition of χ^2; description of quantitative data was realized in the form of M ± m [11]. The results were considered reliable at $p < 0.5$.

RESULTS

In group 1, the nodes were located mainly in the fundus and body of the uterus, which was recorded in 26 patients and accounted for 65.0% of observations ($p < 0.05$). In group 2, the nodes were located intraligamentally (in 40.0% of patients), in the area of fundus and body of the uterus (in 43.3% of women), as well as in the isthmus (in 16.7% of patients). The number of myoma nodes with simple UF varied from 1 to 4 and averaged 1.6 ± 0.14, while with proliferating UF there was a significant increase in the number of nodes to 5–7, with average number of 4.0 ± 0.14 ($p<0.05$). The size of the dominant node with simple UF was 2.6±0.19 cm, with proliferating UF 5.7±0.48cm ($p<0.05$).

It has been proved that the presence of parallel structures in the morphological picture of the biological fluid indicates the presence of hyperplastic processes in patients [12, 13]. In this regard, a significant increase in their percentage in group 2 confirmed the predominance of proliferation processes in this contingent [11]. At the microscope level, proliferation markers — parallel (Fig. 1) and fibrous (Fig. 2) structures are seen in patients with endometrial hyperplastic processes.

The conducted immunochemical study showed that in menstrual blood of the control group, the level of LIP was 3.0–4.8 ng/ml, in group 1 it rised to 4.8–6.4 ng/ml, and in group 2 up to 11.1–14.7 ng/ml. In turn, LIA in the control group was in the range of 35.1–36.8 pg/ml, in group 1 it decreased to 24.0–27.0 pg/ml, and in group 2 up to 20.0–22.5 pg/ml [11, 14, 15].

Correlation analysis in the group of patients with simple UF (group 1) revealed a relationship between lattice structures and levels of LIP (r = +0.3) and LIA (r = +0.28), transitional forms and concentration of LIP (r = +0.27) and dendritic forms and LIA (r = +0.3) [11]. In patients with proliferating MM (group 2), there was a direct relationship between the LIP level and the size of uterus (r = +0.35) according to the ultrasound examination of pelvic organs [15]. The results of the investigation are summarized in Table 1.

At the final stage, to derive a mathematical formula, the binary logistic regression method was used:

$$P = \frac{1}{1 + e^{-z}},$$

where: P is the probability of the event occurring; e — constant value (2.72), which serves as the base of natural logarithm; z — the degree of inverse logarithm is calculated by the formula:

$$z = 2.172\,FS + 2.238\,PS + 1.568\,NN - 10.915$$

where: FS — fibrous structures, PS — parallel structures, NN — the number of nodes [16].

In the case when P was more than 0.5, the risk of developing proliferating UF increased (more than 50%). In this group of patients, at the next stage, the levels of LIA and LIP were analyzed. With an increase in LIP of more than 11.1 ng/ml and a decrease in LIA of less than 22.5 pg/ml, the risk of an event tends to approach 100% [11].

The introduction of this model into clinical practice will enable, with a high degree of probability, to make a prognosis of the proliferative potential of myoma nodes for each individual patient, which, in turn, gives an answer to the question of the advisability of using hormone therapy in the pre- and postoperative period in women who need myomectomy.

Patients with a high risk of developing proliferating UF, for whom myomectomy is indicated, need either isolated or combined pre- and postoperative treatment with the drug class of gonadotropin-releasing hormone agonists in order to suppress proliferative processes. At the same time, for patients with simple UF, a one-stage treatment, including myomectomy, is sufficient, followed by a decision on planning pregnancy.

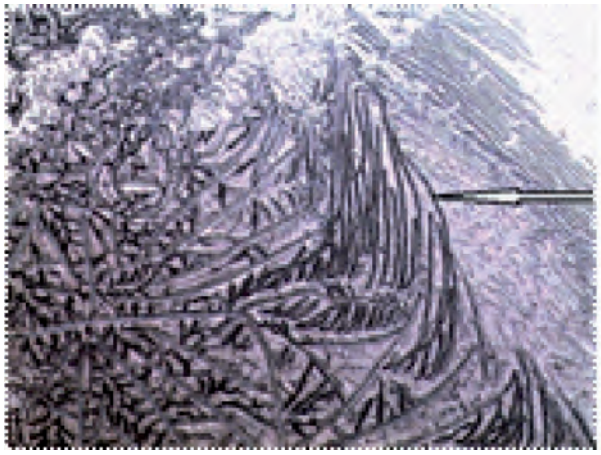

Fig. 1. Parallel structures in an analytical cell. Magnification 100×

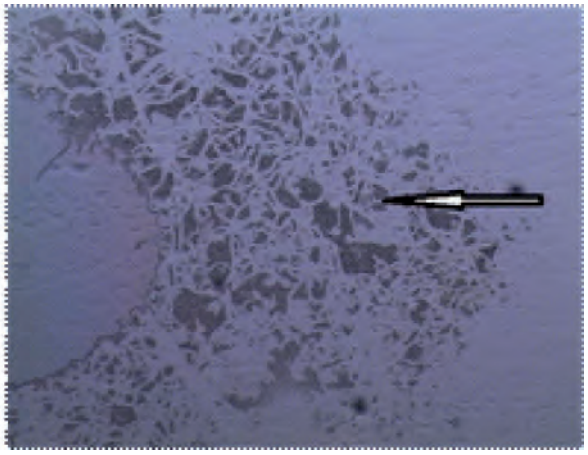

Fig. 2. Fibrous structures in an analytical cell. Magnification 100×

Table 1. Structural elements of the solid phase of the menstrual blood

Isotropic forms	Control group (n = 41), %	Group 1 (n = 40), %	Group 2 (n = 30), %
Dendritic	41,5	47,5	13,31
Transitional	34,2	45,0	3,31
Lamellar	9,8	27,52	3,31
Parallel	2,4	37,52	83,31
Fibrous	0	10,0	50,01

Note: 1) p < 0.05 — significant differences compared to group 1; 2) p < 0.05 — significant differences compared with the control group

CONCLUSION

Therefore, identification of characteristic structural features of menstrual blood in patients with different clinical and morphological variants of the tumor, along with the determination of levels of LIP and LIA, allows not only to make an individual prognosis, to plan the management and treatment of patients, but also ultimately helps achieve the main goal — the implementation of reproductive life planning (RLP). At the same time safety and simplicity of morphological method of biological fluids makes it possible to apply actively this method in an outpatient care facility.

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THE RESULTS OF USING A BIOACTIVE GLASS-BASED COATING BY DEPOSITION ON THE CONTACT SURFACE OF PLATES IN BONE FRACTURES ASSOCIATED WITH TUMORS

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ABSTRACT — The article reports on the results of surgical treatment in 20 patients with bone tumors using a coating containing bioactive glass on the contact surface of the fixation plate. As a result of metallic osteosynthesis of the bones, infectious complications were observed in 1 (5%) patient and tumor recurrence was observed in 2 (10%) patients. Limb function after metallic osteosynthesis ranged from 72.3% to 97.4% depending on the location of the lesion focus. The quality of life of the operated patients increased to 75–85 points. Thus, the use of plates with bioactive glass-based material deposited on the contact surface of the plate, in metallic osteosynthesis for pathological bone fracture or at the risk of pathological bone fracture achieves more effective integration of metal plate with bone. Consequently, a better functional stability of the bone is achieved at the fracture site. Morphological examination of bone biopsy samples from the implantation site of the plate and the plate itself with the deposited bioactive glass-based coating revealed active regeneration of bone tissue. This resulted in an increase in the density of the restored bone. According to the results of histological and morphometric examination it can be stated that deposition of a material containing bioactive glass on the contact surface of the metal plate promotes reparative osteogenesis at the site of bone damage and its morphogenesis of the lamellar type.

KEYWORDS — metallic osteosynthesis, plate, bioactive glass-based (BG) coating, bone tumor, morphological study.

INTRODUCTION

In the treatment of fractures and diseases of the skeletal system, internal (intramedullary and periosteal) and external (pins and nails) fixators are used to ensure the most reliable osteosynthesis of bone. Complete reposition and stable fixation of bone fragments, optimal rate and rhythm of distraction, sparing attitude to osteogenic tissue, good blood supply to the operated limb, the possibility of functional use of the limb, starting from the first days after surgery, represent conditions for reparative regeneration of bone tissue [1, 2]. A study of the reference literature shows the application of autogenous grafts of bone tissue and various bioactive products of decalcified bone and biocomposite matrices, recombinant human bone morphogenetic proteins and ceramics. All the materials including new cellular technologies are used to stimulate osteogenesis, [3, 4, 5]. Over recent years, there has been an active trend in the development of biomaterials aimed at creating composites that replace damaged tissues including bone tissue [6]. In orthopedics, this was facilitated by the development of the major joint arthroplasty industry and the use of bioceramic implants. [7, 8, 9]. Biomaterials used as implants or as temporary fixators for broken bone (periosteal plates, intramedullary nails) can be represented by biotolerant materials (stainless steel and cobalt-chromium alloys), bioinert (titanium and aluminum oxides), bioactive (calcium phosphate ceramics and silicon-based bioglass) [7]. There are no bioactive metals that would accelerate reparative osteogenesis. In orthopedics, chromium-nickel and chromium-nickel-molybdenum corrosion-resistant steels, alloys of cobalt, tantalum, titanium, pure metals — nickel, silver, titanium are most often used for the manufacture of surgical implants [10].

Typical representatives of bioactive materials include bioglass (the most commonly used composition is 24.5% Na_2O, 24.5% CaO, 45.0% SiO_2, 6% P_2O_5, varying the composition, you can change their bioactivity and resorption) and materials based on hydroxyapatite (HA) — $Ca_{10}(PO_4)_6(OH)_2$ (dense and porous ceramics) [11, 12]. Hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) is one of the few bioactive materials that supports bone ingrowth and osseointegration when used in orthopedic implants due to its high biocompatibility.

In the last few years, a special term has appeared in the literature – *biocompatible nanoceramics*, which means nanostructured ceramic material used in medicine to restore (replace) damaged hard tissues [9, 13]. The creation of composite materials based on

biphasic ceramics using various binding components, biologically active substances that would provide osteoinduction of osteoplastic material to form a matrix, on which bone tissue will be formed, is one of the promising areas [14]. The concept of biphasic composite materials in the system "HA – TCF (tricalcium phosphate)" was developed based on the assumption of the possibility of regulating the kinetics of biodegradation by changing the ratio: less (HA) and more (TCF) of soluble phases in one material. Dissolution of the TCF component in body fluid promotes the process of mineralization, and the biological behavior of biphasic ceramics depends on the ratio of HA/TCF [15]. The bioactivity of materials is determined mainly by chemical factors, such as the crystalline phase and molecular structure of the material, as well as physical factors — roughness and porosity of the material surface and the ability to form a chemical bond with the surrounding bone [8, 11]. A number of complex and closely interrelated processes take place on the surface of a bioactive implant. The surface of the material, its biocompatibility is closely related to the adhesion of osteogenic and mesenchymal stem cells on its surface [16, 17]. It is the adhesion as well as the distribution of these cells that will affect their ability to proliferate and differentiate into osteoblasts upon contact with the implant. The latter is crucial in the process of establishing a mechanically strong interface with complete fusion between the implant surface and bone tissue without a layer of fibrous tissue, which is usually formed on the surface of a bioinert metal implant [18, 19, 20, 21]. In this article we report on the use of titanium plates in bone fractures on the background of tumors with a BG-based material, deposited on their contact surface.

Objective:

To show the possibilities of application of periosteal plates with a bioactive glass containging material deposited on the contact surface to improve integration of the plate with bone in pathological bone fractures associated with tumors.

MATERIAL AND METHODS

During the period from 2017 to 2020, in Institute of Traumatology and Orthopedics of NAMS of Ukraine 20 operations on metallic osteosynthesis were performed using periosteal plates with a bioactive glass-based coating deposited on the contact surface of the plate, in pathological fractures or at risk of pathological fractures secondary to tumors of long tubular bones. The average age of patients was 39.2±1.4 years (from 28 to 57 years), among them there were 13 (65%) women and 7 (35%) men.

Metallic osteosynthesis was performed using periosteal plates of different lengths and configurations. Plates (medical steel grade BT6) had the following composition: titanium as the main substance, aluminum content — 3.9/2.8 nm, manganese content — 1.3/1.2 nm, iron content — 0.4/0.1 nm, silicon content — 0.1/0.1 nm, calcium content on the surface of the plate — 0.3 nm, chlorine content — 0.2 nm, sulfur content — 0.1 nm.

Table 1 presents the morphological types of tumors identified during metallic osteosynthesis using plates with a BG-based coating deposited on the contact surface.

Table 1. *Morphological types of tumors identified during metallic osteosynthesis using plates with a BG-based coating deposited on the contact surface*

Morphological types of tumors	Number of cases	%
Metastatic tumors	14	70
Chondroma	3	15
Giant cell tumor of bone	1	5
Solitary myeloma	1	5
Lymphosarcoma of bone	1	5
Total	20	100

The scope of surgery depended on the location of the tumor: patients underwent intraosseous bone resection with the tumor; bone defect in chondroma and giant cell tumor was filled with allograft or material containing bioactive glass; in metastatic tumor, solitary myeloma and lymphosarcoma bone defect was filled with polymethyl methacrylate, after which metallic osteosynthesis was produced using a BG-based coating deposited on the contact surface.

Table 2 presents location and number of cases of metallic osteosynthesis of bones using a BG-based material deposited on the contact surface of the implant.

The use of plates with a bioactive glass-based coating deposited on the contact surface achieves a better integration of the plates with the bone, as well as the fact that in bone tumors, the plates are usually not removed over time.

The material deposited on the contact surface of the plate was an implant material containing bioactive glass — Syntekist Biocomposite (certificate of state registration No. 3653/2005 dated January 28, 2005), which was synthetically produced in the laboratory of the Institute of Materials Science of the National Academy of Sciences of Ukraine by the team of prof. V.A. Dubok.

Implant material — Syntekist biocomposite is a multiphase inorganic material synthetically produced

Table 2. *Location and number of cases of metallic osteosynthesis of bones using a BG-based material deposited on the contact surface of the implant*

Location	Number of cases	%
Distal femur	7	35
Humeral diaphysis	4	20
Tibial shaft	3	15
Proximal tibia	2	10
Distal tibia	1	5
Proximal femur	1	5
Radial shaft	1	5
Femoral shaft	1	5
Total	20	100

by chemical precipitation and ceramic technology. Its phase composition includes bioactive glass — 50–65 wt.%, hydroxyapatite — 14–17 wt.%, whitlockite — 14–17 wt.%, wollastonite — 7–9 wt.%. The chemical composition is shown in Table 3 (in terms of oxides).

Table 3. *Chemical composition of Syntekist biocomposite*

Composition	Na_2O	K_2O	SiO_2	CaO	P_2O_5	Ag_2O
	15,54	0,173	27,58	35,45	21,27	-
Range of permissible composition	15–17	0,1–0,3	25–28	34–37	20–23	0,01–0,08

It is a bioactive and osteoconductive biomaterial, which is available in the form of powders, granules, blocks and implants of complex shape with different activity, dispersion and adsorption capacity, with a significant range of porosity and mechanical properties. The contact surface of the plate was covered with a BG-based material by means of gas detonation deposition. Abrasive treatment of the contact surface of the plate was performed in the gas detonation deposition unit before depositing the BG-based coating; on the surface a surface relief of a given depth was formed, which was equal to the size of osteon and provided a large contact area and adhesion strength between the bone and the plate. Next, the deposition of a layer of the BG-based material has been carried out, which allows to solve the problem of amorphization of the coating. Due to the presence of bioactive compounds in the coating, evenly distributed throughout its thickness, in the process of resorption of the coating in the body, it caused antibacterial, osteoconductive, antiresorptive and other effects throughout the life of the plate (see Fig. 1).

The functional result of the operated limb was determined according to the MSTS Rating scale (Musculo-Sceletal Tumor Staging /System/). Patients' quality of life was determined in points according to the EORTC QLQ-30 questionnaire. Patient survival was determined using the Kaplan-Meier method.

FINDINGS

Of 20 patients, infectious complications were observed in 1 (5%) patient and tumor recurrence was observed in 2 (10%) patients after metallic osteosynthesis for pathological fractures and the risk of pathological bone fractures, In a patient with an infectious complication, after removal of the plate, radical surgical treatment of the wound was performed with removal of all pathologically changed tissues, inoculation of fluid for culture from the place of installation of a plate, washing the wound with antiseptic solutions and installing an active drainage system with antibiotic irrigation.

In 2 patients with tumor recurrence a bone biopsy was performed after plate removal at the point of contact with the BG-based coating deposited on the plate. A biopsy of the metal plate surface was performed followed by morphological microscopic examination of the obtained material. These 2 patients subsequently underwent resection of bone articular segment and joint endoprosthesis replacement due to tumor recurrence.

Functional outcome in the limb (according to the MSTS scale) after metallic osteosynthesis of the distal femur was 82.5%; in the humeral diaphysis — 95.4%, of the tibial shaft — 91.6%; in the distal tibia amounted to 86.8%; in the proximal tibia — 93.2%, in the proximal femur — 72.3%; in the radial shaft — 97.4%; in the femoral shaft — 76.4%.

The quality of life of patients according to the EORTC QLQ-30 questionnaire before metallic osteosynthesis was estimated as 20–30 points, after metallic osteosynthesis it was 75–85 points.

The overall three-year survival of the patients was 46.5 ± 2.9%, and the five-year survival was 26.2 ± 4.1%.

Here is an example from our practice: Patient Ya., 40 years old, considers herself ill since October 2020, when pain and swelling appeared in the distal segment of the left lower leg. According to the patient, the pain intensified within a month, so the patient was forced

Fig. 1. *A metal plate with a BG-based coating deposited on the contact surface*

to walk on crutches. A month later, the patient went to the clinic of the State Institution "ITONAMNU", where X-ray examination revealed a tumor of the left distal tibia (see Fig. 2). In November 2020, the patient underwent an open biopsy of the lesion focus in the distal part of the left tibia. Histopathological findings: giant cell tumor of bone. In December 2020, the patient underwent a intraosseous resection of the lesion focus in the distal part of the left tibia with a tumor and a plastic repair of the defected femur using a BG-based material and extramedullary metallic osteosynthesis of the distal tibia using plate with a BG-based coating by deposition on its contact surface (see Fig. 3). No complications were observed in the postoperative period. The function of the left lower leg (according to the MSTS scale) was 86.8%. There was no tumor recurrence on X-ray in 1.5 months.

In 2 patients with tumor recurrence after plate removal (in 2 months after implantation) during the morphological examination of bone tissue from the area of plate removal and the surface of the metal plate coated with BG-based material we found out: in the subject No.1 bone perimeter was characterized by active osteogenesis. Crystalline granules were detected, which probably represent biocomposite residues after plate removal. Almost the entire surface of the plate was surrounded by newly formed bone tissue, mainly vowen bone. Although in some areas the morphogenesis of lamellar bone tissue has been observed in newly formed trabeculae (Fig. 4). The relative surrounding of the bone defect (at the site of bone trepanation) by newly formed bone tissue amounted to 82.0 (78.3–91.7)%.

In the subject No.2 during morphological examination at the site of the defect, a damaged hyaline cartilage of the articular surface and the metaphyseal plate with replacement by connective tissue was revealed. In the areas where the granules of the biocomposite got into the lacunae of the cortical bone, active

Fig. 2. *Photographs of radiographs (A is a frontal and B is a lateral projection) of the left tibia of patient Ya. with a giant cell tumor of the distal tibia*

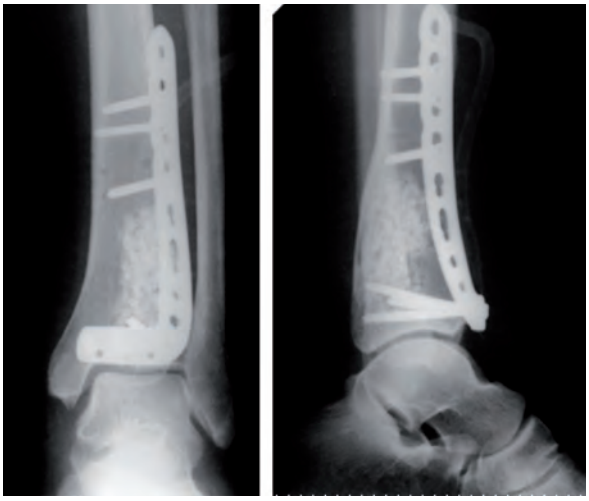

Fig. 3. *Photographs of radiographs (A is a frontal and B is a lateral projection) of the left tibia of the patient Ya. after metallic osteosynthesis for a giant cell tumor of the distal tibia using plate with a BG-based coating deposited on its contact surface. The defect of the bone was repared and filled with the BG containing material*

osteogenesis was detected around this material (Fig. 5). The biocomposite was partially surrounded by connective tissue, mostly an osseous one. Morphogenesis corresponded to the formation of lamellar bone tissue. The relative density of the newly formed bone tissue around the granules of the biocomposite reached 89.6 (72.6–97.8)%.

In the subject No.3 an epiphyseal defect was revealed during the morphological examination, and

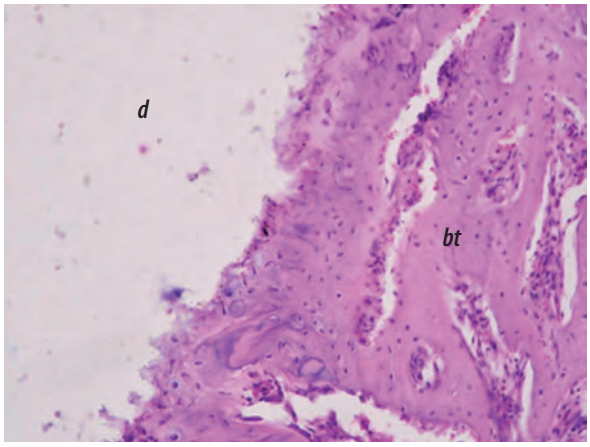

Fig. 4. Active osteogenesis around the metal plate. Note: d — defect; bt — bone tissue. Hematoxylin-eosin, vol. 20, approx. 10

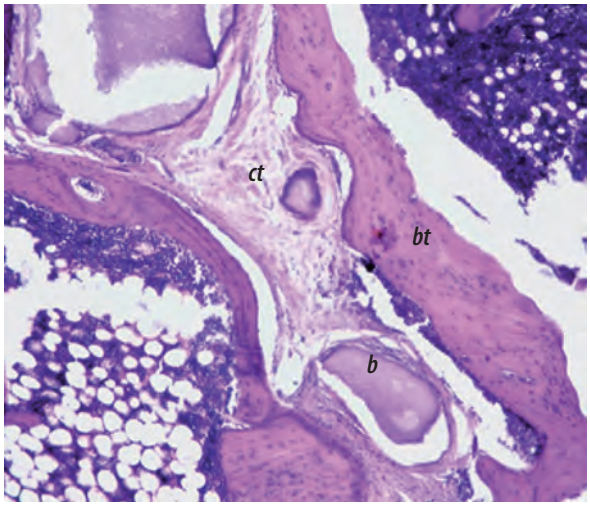

Fig. 5. Active osteogenesis around the biocomposite. Note: b — biocomposite; ct — connective tissue; bt — bone tissue. Hematoxylin-eosin, vol. 10, approx. 10.

the centers of active osteogenesis around the granules of the biocomposite were detected in the cavities of the trabecular bone (Fig. 6). Almost the entire surface of the granules (92.5 (83.5–95.6)%) was surrounded by newly formed bone tissue (the lamellar type dominated). Isolation of the biocomposite from the surrounding morphofunctional bone formations (bone marrow, cartilaginous tissue of the articular surface, metaphyseal plate) was observed.

In the subject No.4 during the morphological examination it was detected that the bone defect was filled with connective tissue. Foci of inflammatory infiltration (neutrophils and macrophages) were detected. The perimeter of the defect was surrounded by newly formed bone tissue, but the boundary between the defect and the bone tissue remained partially filled with connective tissue (Fig. 7).

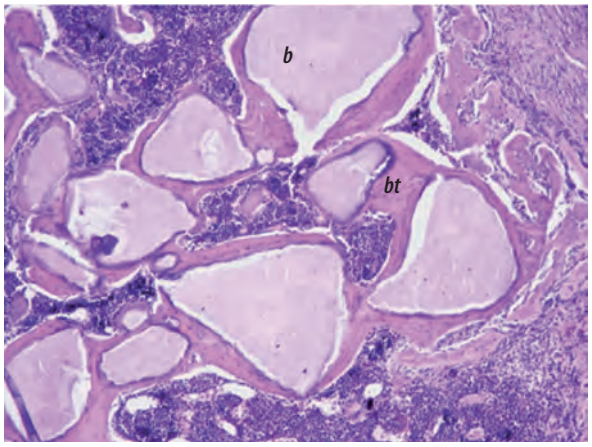

Fig. 6. Active osteogenesis around the biocomposite. Note: b — biocomposite; bt — bone tissue. Hematoxylin-eosin, vol. 10, approx. 10.

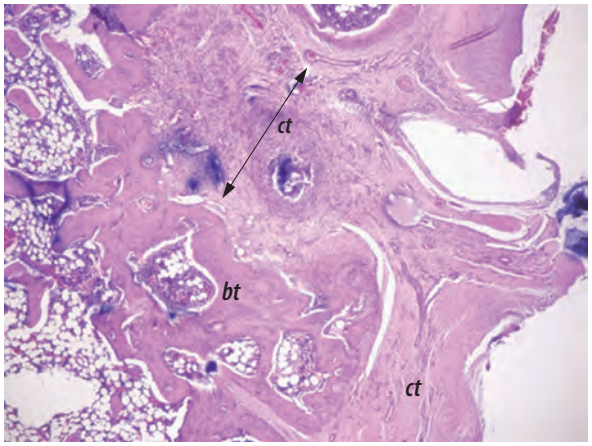

Fig. 7. The bone defect is filled with connective tissue with macrophage infiltration. Note: ←→ defect; bt — bone tissue; ct — connective tissue. Hematoxylin-eosin, a: vol. 4, approx. 10

In the subject No.5, a morphological examination revealed an area of bone defect with biocomposite granules. Osteogenesis around the biocomposite granules was uneven: one part was surrounded by connective tissue, and the other part was totally integrated into regenerated bone tissue (Fig. 8). Newly formed bone tissue in most areas is lamellar.

During morphological examination in the subject No.6 (Fig. 9) the defect was detected at the level of epiphyse, filled with connective tissue, articular and

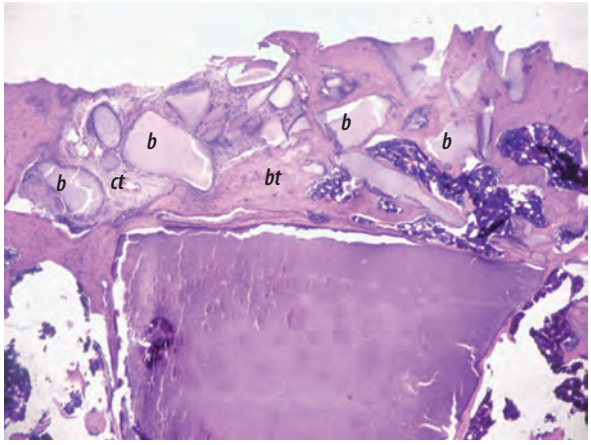

Fig. 8. Heterogeneous osteogenesis around the biocomposite. Note: b — biocomposite; ct — connective tissue; bt — bone tissue. Hematoxylin-eosin, vol. 20, approx. 10

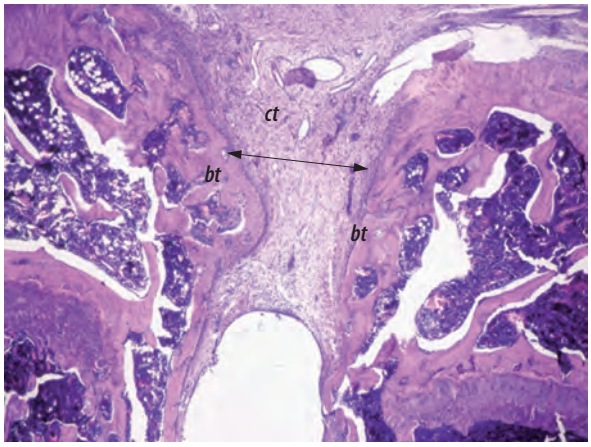

Fig. 9. The defect is filled with connective tissue, active osteogenesis is present. Note: ◄—► defect; bt — bone tissue; ct — connective tissue. Hematoxylin-eosin, vol. 4, approx. 10

metaphyseal hyaline cartilage are deformed. The metaphyseal plate is penetrated by a defect surrounded by newly formed bone tissue, which is lamellar to a greater extent. The level of regeneration was 87.2 (86.7–88.1)%.

RESUSTS

As a result of metallic osteosynthesis in pathological bone fracture or at risk of pathological bone fracture associated with bone tumors, positive treatment results were obtained: limb function ranged from 72.3% to 97.4% depending on the location of the lesion focus, quality of life of operated patients increased to 75–85 points. Morphological examination of bone biopsy material from the site of implantation of the plate and from the plate itself with the deposited BG-based material revealed active regeneration of bone tissue leading to an increase in the density of the restored bone. The results of histological and morphometric examination demonstrate that the deposition of the BG-based material onto the contact surface of the metal plate promotes reparative osteogenesis at the site of bone damage and its morphogenesis of the lamellar type.

CONCLCUSION

1. As a result of application of periosteal plates with deposition of the BG-based material for pathological bone fractures we observed infectious complications in 1 (5%) patient and tumor recurrence in 2 (10%) patients, out of 20 patients with bone tumors.

2. The results of histological and morphometric examination showed that the deposition of the BG-based material onto the contact surface of the metal plate for metallic osteosynthesis promotes more effective integration of the plate with bone, which leads to a greater functional stability of the bone at the fracture site.

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RESULTS OF SURGICAL TREATMENT OF PATIENTS WITH PANCREATIC INJURIES

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ABSTRACT — The analysis of 12 cases of surgical treatment of young male patients, with injuries of the pancreas is presented. By the nature of the injuries, half of the patients had gunshot injuries, and 4 had blunt injuries, 2 patients with stab and slash wound. All injuries were combined; with severity grades I–III according to AAST classification. All patients underwent laparoscopic procedures. In one patient distal pancreatectomy was performed, whereas in 7 patients the appropriate sutured closure and in 4 patients drainage closure were performed. In one patient, we used a gastric transplant on a vascular pedicle. This case is of particular interest, so we presented it separately.

KEYWORDS — pancreas, surgery, injury, laparoscopy.

INTRODUCTION

According to various authors, the lesion on the pancreas is quite rare and occupies the last place along with intestines, spleen, liver and kidneys injuries which represents less than 5% of abdominal trauma [1,2,3,4,5]. The existing surgical tactics are rather contradictory. Some authors consider it possible to perform primary repair operations, while others are opposed to this approach [6]. However, the experience of domestic and foreign authors shows that the risks and complications that are usually associated with reconstructive surgery may be exaggerated. Nevertheless, the question of safety and feasibility of pancreatic resection requires further study. The difficulty and uncertainty of complete hemostasis is still one of the main factors determining the restrained attitude of surgeons to resection.

PURPOSE OF THE WORK

The management of pancreatic trauma is complex. The purpose is to report our experience in the management of pancreatic trauma with consideration of morbidity and mortality.

MATERIALS AND METHODS

All patients hospitalized between 1998 and 2017 for pancreatic trauma in the clinic of the Department of Surgical Diseases of the Faculty of Dentistry of Astrakhan State Medical University were investigated. Traumatic injuries of the pancreas were classified according to the American Association for Surgery of Trauma (AAST) in five grades [3]. We observed 12 patients with pancreatic injuries. There were only male patients among them. They were distributed according their age as follows (Table 1).

According to the nature of injuries, half of the patients were with gunshot injuries, 4 — with blunt injuries (Table 2).

All injuries were combined, the localization of wounds and their size are presented in Table 3.

The size of the wounds from 1 to 2 cm prevailed among all the patients and amounted to 5 (42%) people. The most frequent lesion was to the pancreatic body — 6 (50%) patients.

RESULTS AND DISCUSSION

In seven patients, the wounds were sutured with interrupted stitches, in one case there was bleeding, as a result of which relaparotomy was performed. The bleeding was stopped by suturing the gland tissue. In one patient, in addition to the nodal suture, the peritoneum was used as a lining material, and in another, a strand of the omentum on the vascular stem was used. In one case, the gland wound was sutured with an *8-shaped suture*. In one patient a resection of the pancreatic tail with suturing of the resulting *U-shaped* suture wound was performed. In one patient, we used a gastric graft on the vascular stem. When forming a gastric graft, a serous-muscular flap with the right gastroepiploic artery was cut out from the greater curvature of the stomach. This case is of a particular interest, so it is worth being presented.

Patient P. 17 years old, IB No. 3743, was admitted to the clinic with diffuse peritonitis a day after being injured in a motor vehicle accident. The patient underwent laparoscopy, during which a sanious liquid with flakes and jelly-like clots was found in the abdominal cavity. Midline laparotomy was performed. There was a moderate amount of sero-hemorrhagic exudate in the abdominal cavity. There were multiple *stearic plaques*

Table 1. Distribution of patients by age

	males	In total
Under 20 years old	3 (25%)	3 (25%)
From 21 to 30 years old	4 (33,3%)	4 (33,3%)
From 31 to 40 yaers old	5 (42%)	5 (42%)
In total	12	12

Table 2. The distribution of the patients according to the nature of injuries

The nature of injuries	Blunt trauma	Stab and slash wound	Gunshot wounds	In total
males	4 (33,3%)	2 (17%)	6 (50%)	12

Table 3. Localization and size of wounds

Localization of wounds	Up to 1 cm	From 1 to 2 cm	From 2 to 3 cm	8 cm	The whole surface	In total
The whole pancreas					1 (8,3%)	1 (8,3%)
The body of the pancreas	1 (8,3%)	2 (17%)	3 (25%)			6 (50%)
The head of the pancreas		3 (25%)				3 (25%)
The tail of the pancreas			1 (8,3%)	1(8,3%)		
In total	1 (8,3%)	5 (42%)	4 (333%)	1 (8,3%)	1 (8,3%)	12

Table 4. Characteristics of patients who underwent laparotomy

Patient (n)	Mechanism of Injury	AAST grade	Surgical management	Postoperative complication
1	Penetrating trauma	3	Distal pancreatectomy	None
2	Motor vehicle accident	3	Suturing the wound+ gastric graft on the vascular stem	None
3	Penetrating trauma	1	Suturing the wound	Pancreatic fistula
4	Motor vehicle accident	1	Suturing the wound	None
5	Motor vehicle accident	2	Suturing the wound	acute pancreatitis
6	Motor vehicle accident	2	Suturing the wound	None
7	gunshot wound	2	Suturing the wound	peritonitis
8	gunshot wound	2	Suturing the wound	None
9	gunshot wound	2	Drainage	acute pancreatitis
10	gunshot wound	2	Drainage	None
11	gunshot wound	2	Drainage	peritonitis
12	gunshot wound	2	Drainage	intra-abdominal bleeding

on the large and small omentum. The omentum sac was opened, and a large amount of cloudy effusion was released. The revision revealed a large retroperitoneal hematoma extending to the small omentum and mesenteric root. After opening the posterior peritoneum above the pancreas, a complete transverse rupture of its body was found at the borders of the head and body. The gland was hyperemic with areas of necrosis at the site of rupture. An attempt to isolate the Wirsung duct failed due to rapid infiltration of glandular tissues and of uprisal bleeding. The bleeding was stopped. Necrotic tissues were removed and the wound surface of the distal part of the pancreas was covered with a gastric graft. After the mobilization of the pancreatic body, it was possible to detect the Wirsung duct with a diameter of up to 0.8 cm. A chlorvinyl tube was inserted into its lumen, which was removed in the left hypochondrium. Interrupted stitches were applied to the proximal part. A month after the discharge, the patient developed clinical signs of acute adhesive intestinal obstruction. Relaparotomy was performed. During the revision, the cause of the obstruction was a tube that drained the duct, and good engraftment of the gastric graft was noted in the area of the previous operation. The gastric graft had a normal color, without signs of swelling. Adhesions were dissected, intestinal obstruction was eliminated. It was decided to transfer external drainage to the internal one. Pancreaticogastrostomy was performed. The patient made an uneventful postoperative recovery.

In this case, we observed good engraftment of the gastric graft against the background of existing diffuse peritonitis, that is, in conditions of infection of the abdominal cavity. The distal part of the gland was sutured with interrupted stitches. Organ-preserving surgery was also performed in order to avoid unnecessary splenectomy and possible violation of the endocrine function of the pancreas.

During repeated surgery for intestinal obstruction, the external pancreatic fistula was transferred to the internal one, not only because of the high degree of physiology of this method of drainage, but also for reasons of preventing repeated adhesive intestinal obstruction.

The postoperative period was difficult in 6 (50%) patients: 2 (17%) of them had acute pancreatitis; 1 (8,3%) pancreatic fistula; 2 (17%) diffuse peritonitis; 1 (8,3%) bleeding.

The findings reveal two lethal cases among our patients. In the first patient there were acute post-traumatic pancreatitis, pancreatic necrosis, diffuse purulent-fibrinous peritonitis, multiple organ failure and intoxication. The second patient had a complete rupture of the pancreatic body. He developed diffuse purulent-fibrinous peritonitis, multiple organ failure, and cerebral oedema.

CONCLUSION

Operative management of pancreatic trauma may lead to a higher mortality. This must not be necessarily related to the pancreas injury alone but due to the associated injuries such as liver, spleen and vascular traumas are more likely to occur with severe outcomes.

As stated above, pancreatic injuries are quite rare. Nevertheless, they are of particular interest, as they may develop severe and sometimes even fatal complications. The gastric graft acts as a barrier to prevent the leakage of pancreatic fluid. It promotes hemostasis, due to the improvement of vascularization and nutrition of pancreatic tissues. Moreover, the gastric graft contributes to a more rapid subsidence of pancreatitis, whose manifestations are clinically shown. It should be noted that the postoperating period was uneventful and it required less hospital stay after the surgery. The presented data and the methods of performing a gastric graft on the vascular stem in cases of pancreatic damage can be recommended for clinical practice.

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COMPLICATIONS OF OPEN SURGICAL PROCEDURES IN PATIENTS WITH PANCREATIC CYSTS

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ABSTRACT — The incidence of pancreatic cysts is constantly growing due to an increasing number of patients with a history of pancreatitis. Furthermore, complications after surgical interventions for pancreatic cysts occur in 30-40% of patients.
OBJECTIVE: to analyze the complications in patients with pancreatic cysts after open surgery.
We observed 68 patients who underwent open surgical interventions: cystogastrostomy and cystojejunostomy. All patients with pancreatic cysts were divided into 2 groups depending on the type of procedure. In 27 patients (29%) of the first group cystogastrostomy and in 41 patients (81%) of the second group cystojejunostomy was performed.
In 1st group complications were diagnosed in 8 cases (29.6%): postoperative pancreatitis (5 — 18.5%), cyst recurrence (3 — 11.1%). In the second group complications occurred in 7 patients (17.1%): postoperative pancreatitis in 4 cases (9.8%), recurrent cysts in 3 cases (7.3%).
Thus, cystojejunostomy is less associated with complications than cystogastrostomy.

KEYWORDS — pancreatic cyst, chronic pancreatitis, cystogastrostomy, cystojejunostomy.

RELEVANCE

Acute pancreatitis is considered the single most frequent gastrointestinal cause of hospital admissions in the United States. Chronic pancreatitis being lower in incidence noticeably worsens patients' quality of life. In recent decades researchers worldwide have witnessed an increase in patients with a history of pancreatitis, and as a result, the presence of pancreatic cysts in this group of patients [1, 2, 3]. According to scientific literature the process of formation of pancreatic cysts is often accompanied by the appearance of severe complications: mechanical jaundice, perforation, suppuration of cysts, peritonitis, bleeding, etc. [4, 5, 6]. After open surgical interventions complications occur in 30–40% of cases. Those complications are quite variable: from the most severe, such as bleeding, peritonitis, abscesses, to the milder, that could be stopped conservatively or with the help of minimally invasive procedures — postoperative pancreatitis, recurrent pancreatic cysts. These complications are often associated with high pressure on the anastomosis caused by increased pancreatic secretion, as well as technical intraoperative difficulties [7, 8].

Objective: to evaluate complications in patients with pancreatic cysts after open surgical procedures.

MATERIAL AND METHODS

The work has been carried out in surgical departments of Penza Regional Clinical Hospital named after N. N. Burdenko. The study involved 68 patients who underwent open surgical interventions: cystogastrostomy and cystojejunostomy. There were 12 women (20.7%) and 56 men (79.3%). The age of patients ranged from 22 to 74 years. The average age was 57±9.8 years. Pancreatic cysts were identified: in the tail of the gland in 6 patients (10.3%), in the body of the pancreas in 8 patients (13.7%), in the head of the pancreas in 54 patients (75.7%).

At the first stage 59 patients (84.4%) underwent external drainage of pancreatic cysts under ultrasound control. The remaining 6 patients (15.5%) did not undergo this procedure.

Indications for external drainage of cysts were: an increase in the size of the cyst more than 50 mm, a loose, unformed cyst wall, signs of infection of cysts, as well as the severity of the patient's condition.

For diagnostic purposes in the patients with pancreatic cysts the following general clinical and laboratory tests were performed: blood type and Rh factor, common blood test, biochemical blood test, general urine test, urine diastasis, coagulogram; instrumental examinations: electrocardiography, ultrasound examination of the abdominal cavity and retroperitoneal space, fibrogastroduodenoscopy, computed tomography of the abdominal cavity and retroperitoneal space, X-ray examination of the chest and abdominal cavity (fistulography after drainage of pancreatic cysts was performed to identify the connection with the pancreatic duct), magnetic resonance imaging of the abdominal cavity and retroperitoneal space.

All patients with pancreatic cysts were divided into 2 groups, depending on the type of surgery performed. Group 1 included 27 patients who underwent cystogastrostomy (29%), group 2 included 41 patients where cystojejunostomy was done (81%).

Indications for cystogastrostomy and cystojejunostomy were: the size of the pancreatic cyst more than 60 mm, the formation of the pancreatic cyst wall, as well as the presence of a proven instrumental connection between the cyst and the pancreatic duct.

Cystrogastrostomy was performed in patients with a pronounced adhesive process in the abdominal cavity, with the fusion of the cyst wall with the posterior wall of the stomach. Cystojejunostomy was performed in all remaining cases.

RESULTS AND DISCUSSION

In group 1 after surgery, complications were diagnosed in 8 cases (29.6%): cyst recurrence in 3 patients, postoperative pancreatitis in 5 patients.

In 5 cases (18.5%), the development of postoperative pancreatitis was revealed with clinical manifestations on the 4th–5th day after surgery. After conservative therapy, patients were discharged in 15–16 days in a satisfactory condition. The following scheme of conservative treatment has been used: non-narcotic analgetics (ketorol 1.0 v/m), NSAIDs (ibuprofen 3.0 v/m), antispasmodics (no-shpa 2% — 2.0, platyphylline 0.2% — 1.0 v/v), infusion therapy in the volume of 20–30 ml/kg of weight was used; drugs that reduce pancreatic secretion (atropine 0.1%–1.0 i/v), as well as inhibitors of protease activity (kontrikal 20,000 units 2 p.i/v or gordox 100.000 units).

In the long-term postoperative period in 3 cases (11.1%) the cyst recurrence was detected (after 4 months during a routine ultrasound examination). No clinical manifestations of pancreatitis were noticed. The level of amylase activity in all cases was within the normal range. The size of the liquid collector in the first case was 18 mm, in the second — 29 mm, in the third — 38 mm. Dynamic follow-up at the surgeon's place of residence is recommended.

Complications occurred in 7 patients (17.1%) in the 2nd group. Postoperative pancreatitis was observed in 4 patients (9.8%). In the course of treatment (conservative therapy) acute pancreatitis was stopped on the 12th–16th day from the moment of hospitalization. Recurrence of a pancreatic cyst was detected during a routine ultrasound examination in 3 patients (7.3%) in the long-term postoperative period (from 4 to 6 months after surgery). Infection of the cyst cavity occurred in one case. In the postoperative period this patient suffered from acute pancreatitis. During the next hospital readmission, the liquid formation in the projection of the tail of the pancreas with dimensions of 75×62×67 mm was revealed. The cyst cavity was drained under ultrasound control, and cloudy contents with a fetid smell were obtained. After that procedure no clinic of acute pancreatitis were revealed.

After the surgical treatment in the second group of patients the length of hospital stay was maximum 16 days, minimum — 7 (12 ± 1.4 days). In the first group the period of hospital stay was 15 ±1.35 (from 12 to 19 days).

Thus, the total number of complications in 1 group was 29.6%, where postoperative pancreatitis was detected in 18.5%, and recurrent pancreatic cysts — in 11.1%. In 2 group patients, complications were observed in 17.1%: postoperative pancreatitis (9.8%) and recurrent pancreatic cysts (7.3%). The duration of treatment in patients after cystograstrostomy was 15 days, after cystojejunostomy — 12 days. There were no fatal outcomes after surgical interventions in patients with pancreatic cysts.

CONCLUSION

We consider it more appropriate to perform Roux-en-Y cystojejunostomy on a disconnected loop, since the postoperative period with such a surgical intervention is accompanied by fewer complications (by 1.7 times — p=0.017) and a shorter length of hospital stay. The median postoperative LOS after cystogastrostomy is 1.25 times more than after cystojejunostomy.

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LITERATURE REVIEW METHODS FOR PROSTHETIC MESH FIXATION IN SURGICAL REPAIR OF INGUINAL HERNIAS

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ABSTRACT — The article is devoted to the evolution of fixation methods for synthetic mesh endoprostheses in surgery of inguinal hernias. We have analyzed the data on failures and complications of synthetic implants. We paid a special attention to available techniques, such as endoprostheses fixation without sutures as well as to the fixation using various types of glue. Our review is based on its own experimental and clinical data as well as the data from world's best hospitals. We found that the use of fibrin glue frequently leads to formation of seromas and hematomas, whereas albumin-glutaraldehyde glue may result in abscesses and pyogenic inflammatory infections of surgical sites. The authors pointed out the importance of further research for the optimal fixation of the prosthetic mesh in hernia repair.

KEYWORDS — inguinal hernias, hernia prosthesis, mesh implants, methods of fixation, postoperative complications, surgical glue, surgical hernia repair.

INTRODUCTION

In the conditions of modern inguinal hernia surgery, there is a tendency to focus on the methods of surgical interventions and types of mesh implants without considering the multiple disadvantages of conventional methods for mesh fixation. Dozens of experiments carried out over the past 20 years proved the existence of a pronounced inflammatory process along the periphery of implanted prosthesis (Graft-versus-Host disease), which onset can reach 5–10 years after hernioplasty [1]. As a result, there are many complications emerging after inguinal hernia repair (acute and chronic pain, sense of discomfort, paresthesia, neuralgia, up to the inflammation of the implants and their failure) [2].

At the end of the twentieth century, a lot of studies were published, where the analysis of the causes of recurrence after hernia repair was carried out. The main reason for their development was the migration of the implant, so the need for secure fixation of the mesh was reported [3]. In the literature, there are several reasons for the migration of implants — mechanical (primary) and those caused by dystrophic changes in the tissues surrounding the implant (secondary) [4]. Primary ones arise mainly because of inadequate fixation of the mesh implant [4].

In terms of modern hernia repair technologies, many questions come up about the possibilities of sutureless fixation and their advantages compared to traumatic (penetrating) fixation. The method of sutureless fixation was introduced into clinical practice of premises of our department in 2015; however, the experimental study of this technique had been conducted in the United States and European countries since the beginning of the second decade of the 21st century and has been continued up to the present day [5]. The possibilities of body tissues connection still causes much discussion, and the scientific search is going on [6].

Chronic pain after hernioplasty occurs in addition to many external factors, from the body's inflammatory response to the implant and the involvement of sensory nerve endings up to a psychoemotional state of the patient. However, the leading cause of neuralgia, pain, and paresthesia after inguinal hernia repair is the involvement of sensory nerve endings in the suture area or in inflammatory tissues after placement of hernia implant [7].

METHODS

We selected the published data, including PubMed and Web of Science databases on the influence of the method of fixing mesh prostheses in the surgical treatment of hernias. 118 sources were analyzed from 2003 to 2020, and 27 sources included in the article.

RESULTS OF SELECTION AND CONTENT OF THE REVIEW

Among all the methods of sutureless fixation, ParieteneProGrip implant (Sofradim, France) can be

pointed out. It consists of a mesh with micro-grips represented by polypropylene fibres and resorbable polylactic acid micro hooks on its lower surface, thanks to which it is fixed ("Velcro") [8]. The results of the first experience of using this type of mesh in the Russia by Protasov A.B. are very positive: the number of complications decreased by sixfold and duration of the operation declined by 1.4 times [9].

There is also a number of rigid meshes with shape-memory features, for example Hertramesh (Herniamesh, Italy), which, according to the authors, do not require any fixation. However, during the studies the authors noticed significantly more frequent development of various kinds of complications (chronic pain, foreign body sensation, neuralgia and paresthesia) in comparison to lightweight meshes [10].

The use of adhesive compositions for mesh fixation during the inguinal hernia surgical repair can be particularly highlighted [11]. Before the start of our first publications (Chair of Surgery named after N.D. Monastyrskiy) on the use of adhesive compositions [12, 13] there were few publications on this issue in the Russia, and most surgeons had no idea about the use of this method of fixation. With the beginning of development and the first demonstrations, several dozen publications appeared dealing with the use of various kinds of glue mesh fixation methods.It confirms the value and need for further development of this method of fixation in our country. According to the foreign literature data, the inflammatory process in the mesh area after the use of adhesive compositions is much less obvious at the edges [14, 15]. In the modern world, there are various types of glue used for inguinal hernia repair; the most common is Fibrin glue that is used in traditional methods of inguinal hernia repair. However, there are reports of the possibility of its use in endovideosurgical methods of hernioplasty [16].

In 2014, The European Hernia Society did not include direct instructions on the use of adhesive compositions for mesh fixation in the EHS Guidelines for the Treatment of Inguinal Hernia, but it was mentioned that this technology can lead to a decrease in the risk of chronic pain development, but there is not enough studies on this subject [17].

The use of cyanoacrylates for inguinal hernia surgical repair is of great interest [21]. In 2007 Austrian authors carried out an experiment demonstrating the undesirability of using cyanoacrylates in hernioplasty, as, in their opinion, the glue was not resorbed and it caused persistent deformation of the anterior abdominal wall in the area of its application. In the next experiment that was carried out more than 5 years later, the complete safety of cyanoacrylate use and their complete resorption after 40–50 days from application was proved; the authors associate the unsatisfactory results of the Austrian colleagues with applying too much glue [22]. Up to date, two works have been published concerning the use of cyanoacrylate glue (Switzerland, Finland, 2011). The first study compares the use of lightweight meshes and cyanoacrylate glue in traditional hernioplasty with the same lightweight meshes and absorbable sutures. The results indicated 2 times higher percentage of recurrences but at the same time 2 times lower level of chronic pain while using the glue fixation method [23]. The second study, published 5 years later, showed an identical level of recurrence and chronic pain development; however, the authors noted the presence of wound suppuration while using glue fixation — in 3.4% of cases.

CONCLUSION

Based on the experiment data, it can be concluded that:

— the use of traumatic fixation methods (for example, sutures), reduces mesh contraction in the postoperative period [18];

— the use of fibrin glue is associated with fewer postoperative complications such as seromas or hematomas [19];

— the use of albumin-glutaraldehyde glue causes formations abscess and other pyoinflammatory diseases in the prosthetic mesh area [20].

Considering the above, it is rather difficult to estimate the possibility and safety of using glue fixation methods for hernia repair; this issue requires further study and a series of experiments to make any conclusions [24]. Of course, it is necessary to conduct further research on this issue and major clinical experimental studies [25].

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EXPERIENCE OF ENDOVASCULAR EMBOLIZATION FOR RECTAL BLEEDING IN PATIENTS WITH RECTAL CANCER

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ABSTRACT — The article is devoted to the most dangerous complication of colorectal cancer — bleeding. The clinical group included 32 patients with stages 3–4 of the disease. Endovascular embolization by microspheres was performed in 18 patients. The bleeding was successfully stopped in all operated patients. When monitoring the main laboratory blood values in the postoperative period, the development of a systemic inflammatory reaction was not noted. Flexible rectosigmoscopy performed after endovascular embolization did not reveal a single case of necrosis of the intestinal wall above and below the tumor. In order to prevent intestinal ischemia, it is advisable to introduce microspheres in a volume of ⅓ from the circulatory channel of the embolized area. Endovascular embolization by microspheres is an effective method of hemostasis in patients with colorectal cancer complicated by bleeding.

KEYWORDS — colorectal cancer, rectal cancer, rectal bleeding, endovascular embolization, endovascular hemostasis, microspheres.

INTRODUCTION

Colorectal cancer (CRC) is the second most common cause of cancer death in the United States. The incidence rate between 2012 and 2016 ranged from 30 to 45.7 per 100,000 people. Among persons younger than 50 years old, the incidence of tumors of the proximal and distal colon, as well as the rectum, increased by about 2% annually. Given the increasing incidence of CRC in younger patients, in 2018, the American Cancer Society made a qualified recommendation to reduce the age at which patients should begin CRC screening to 45 years and older. It was extrapolated that in 2020, approximately 147,950 people will be diagnosed with CRC in the US, and 53,200 will die from the disease, including 17,930 cases and 3,640 deaths among persons under the age of 50 [1].

Intestinal bleeding, as a complication of CRC, was noted in 26.8% of patients. The complication is usually associated with the breakdown of tumor tissue or germination and arrosion of intestinal wall vessels. In 21.6% of the total number of observations of occult gastrointestinal bleeding, their source is localized in the rectum, in 11% of the total number of observations, bleeding is associated with carcinoma. Profuse bleeding occurs in 2–5% of all cases of rectal cancer (RC) [2, 3, 4].

RC complicated with continued intense bleeding and pronounced anemia poses challenges for doctors of general surgical practice in preparing the patient for surgery, often with low efficiency of blood-substituting measures. Under certain circumstances you have to refuse to perform neoadjuvant exposure. Postoperative lethality and the frequency of complications in patients with RC operated at the bleeding level is higher than in those operated as planned. In primary resections with anastomosis for complicated RC, lethality varies from 4.3 to 8.9%, the frequency of postoperative complications varies from 21.7 to 89%. The five-year survival rate in these patients is 60.9% versus 74.6% in those operated in the absence of complications [5, 6].

The purpose of the work was to summarize the experience of endovascular embolization in 18 patients with RC admitted to a general surgical hospital with bleeding.

MATERIAL AND METHODS

In clinical group there were 32 patients with RC T3-4NxM1. In our studies, we adhered to the 7th revision of the TNM tumor classification of the International Cancer Union. 16 (50.0%) men and 16 (50%) women. The age of patients ranged from 60 to 73 years, averaging 65.8 ± 3.1 years. The inclusion criteria were: the presence of a powerful collateral from the right branch of the upper rectangular artery to the lower sigmoid artery during angiography. Exclusion criteria were: signs of intestinal obstruction, history of other oncological diseases, pronounced aortic atherosclerosis, concomitant blood diseases (thrombophilia), sub- and decompensation of diseases of the cardiovascular, respiratory system, liver and urinary system.

At the first stage of treatment, hemostatic therapy was carried out for 2 hours. With continuing bleeding, a decision was made to conduct diagnostic aortography of the abdominal aorta to identify the source of bleeding. In 12 patients, contraindications to endovascular artery embolization were identified due to the features of angioarchitectonics: pronounced atherosclerotic lesion with aortic calcinosis at the site of withdrawal of the inferior mesenteric artery in 5 patients, pronounced atherosclerotic lesion of the inferior mesenteric artery with calcinosis from the mouth in 3 patients, inflection of the mouth of the lower mesenteric artery in one patient which could lead to dissection of the vessel when conducting the catheter to its distal sections, an additional vessel connecting the rectal artery basin and the sigmoid arterial arcade bearing the danger of non-target embolization of healthy organ vessels in 3 patients. Two patients refused to perform an endovascular intervention. In these patients, bleeding stop continued to be carried out by conservative means. Embolization was performed on the Philips Allura CV 20 angiographic unit. Femoral 5-6Fr introducers were used for vascular access. Selective catheterization of the inferior mesenteric and superior rectangular arteries was performed by a 4-5F catheter. As emboli, microspheres of HepaSphere of Biosphere Medical (France) were used. The choice of emboles is due to the possibility of their saturation with a cytostatic solution, which in our opinion is promising with the further development of the technique.

The volume of embolisate administered was determined according to the method we developed and was ⅓ of the volume of the arterial circulator bed in the area of selective installation of the catheter for embolization. After embolization, arrest of rectal bleeding was monitored clinically and laboratory. Flexiblerectosigmoscopy was performed in dynamics.Attentionwas paid to the presence of hemostasis, superficial necrosis of the tumor, and the condition of the rectal mucosa in the immediate proximity of the tumor. After pre-surgical preparation, all patients were referred for specialized combined RC treatment.

RESULTS AND DISCUSSION

Treatment results were evaluated by the quality of hemostasis, systemic inflammatory response and rectal mucosa conditions above and below the tumor. Hemostasis was achieved in 100% of cases in all 18 patients. According to the results of flexible rectosigmoscopy, the intestinal mucosa above and below the tumor was unchanged. The blood hemoglobin level in the clinical group was reduced and averaged 74.1±11 g/l. In the largest number of observations, blood hemoglobin was distributed in the range of 70–90 g/l (61.1%, n = 11). To assess the degree of inflammation in the body, all patients had their leukocyte intoxication index (LII)determined.On the 1 and 2 day after the operation, patients'LII, compared with the initial value, was increased to 1.2 ± 0.08 and 0.99 ± 0.07. In subsequent stages of observation, the LII decreased to normal values. A statistically significant decrease in total protein content in the blood compared to the original value was observed on days 1 and 2 after surgery. The total blood bilirubin in the first day after the operation increased, but remained within normal limits throughout the observed stage. There were no significant changes in the urea content in the blood of patients in clinical groups. By 3 days after the operation, the blood urea level decreased, probably due to infusion therapy. Thus, the study of the dynamics of laboratory indicators in patients after endovascular embolization of the tumor due to continued bleeding did not lead to a systemic inflammatory reaction.

CONCLUSION

According to the results of the postoperative observation, it can be concluded that endovascular embolization is an effective method of hemostasis in this category of patients.Theintervention does not lead to complications, does not cause a systemic inflammatory reaction, does not complicate the course of the underlying disease in the early postoperative period and is easily tolerated by patients. Performing a flexible rectosigmoscopy after endovascular embolization did not reveal a single case of necrosis of the intestinal wall above and below the tumor. In order to prevent intestinal ischemia, it is advisable to introduce microspheres in a volume of ⅓ from the circulatory channel of the embolized area.

Contributors:

Alexander Khitaryan — designed the study, performed the analysis and interpreted the results; ***Alexander Murlychev*** — conducted review of the literature and collected the data; ***Alexey Orekhov*** — performed the analysis and interpreted the results; ***Sergey Kovalev*** — collected the data and performed the calculations; ***Vyacheslav Mikhailichenko*** — performed the analysis and interpreted the results; ***Dmitry Parshin*** — wrote the manuscript.

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PRECLINICAL TRIALS FOR ADVANCED CHITOSAN-BASED COATINGS IN TREATING PURULENT WOUNDS

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ABSTRACT — Wound healing is a complex set of body responses to damaged tissues, which manifests itself through local destructive-inflammatory changes and general reactions. There are numerous coatings used currently in clinical practice to treat wounds. These coatings serve to develop a wet anti-bacterial environment, which is essential to facilitate the healing process. This work focuses on studying multifunctional coatings, which are based on chitosan, a biocompatible polymer featuring wound-healing properties. The distinctive feature to be found in chitosan fibers is their density and the orientation of pores. The coatings within this study were tested on scalped full-layer skin wounds of male Wistar-Kyoto rats and male rabbits. The sample with a dense structure and low biological resorption was found to be effective in performing the function of a framework, as well as in ensuring proper drainage at the affected area. This is important when treating purulent wounds.
The experimental sample with a high degree of adhesion and a shorter biodegradation life can be recommended for treating wounds with no purulent-inflammatory issues, for granulating wounds, as well as a drug carrier-matrix. The studied wound coatings have revealed their medical efficiency at the preclinical stage (in vivo). Using wound coatings with specified structural and functional features would allow making a reasonable choice when selecting a coating depending on the stage of wound healing course.

KEYWORDS — chitosan; purulent wound; infected wound; wound process; wound coatings; wound treatment; preclinical tests; regenerative medicine; tissue reconstruction.

INTRODUCTION

One of the urgent issues faced currently by medicine is the treatment of patients with injuries, burns, and various skin defects (e.g., trophic ulcers, bedsores, infected postoperative wounds, etc.). All this indicates the need to develop highly effective wound-healing medicines with antimicrobial properties [1–3].

The main factors that impede epithelialization and granulation include tissue dystrophy, oxidative damage, wound moisture content imbalance, infections and other complications affecting the area of surgical resection, injury or burn. The development of scar tissue or other structural changes at the wound will reduce the patient's life quality. Nowadays, there are certain techniques available, which can help facilitate the wound healing process as well as improve the structural and functional properties of the newly developing tissue. Most of such techniques involve wound coatings that differ in their composition and functional features [4–6, 25–27].

The data available in respective literature reveals that purulent-inflammatory processes affecting soft tissues are rated among the most common issues in the entire body of surgical morbidity. The pathologies in question account for 35–45% of all surgical cases, while purulent complications, which affect the post-operative wound area, have been observed in 33–38% of all patients. Moreover, as far as outpatient clinical practice is concerned, soft tissue infections constitute the predominant pathology within the total structure of primary surgical patients [7].

The physical methods, which are used to treat wounds nowadays (vacuum treatment of wounds, ultrasonic cavitation, wound treatment with a pulsating jet, etc.) do add to the positive treatment outcome. However, even with all these advanced methods, the treatment of purulent and infected wounds can never be effective without wound dressing [8].

In modern conditions, the physical and hygienic properties featured by materials based on natural cotton no longer meet clinical specialists' needs. Given that, there has been a wider use of dressings made with advanced technologies and featuring a set of the following properties: inertia in relation to biological tissues; minimal mechanical trauma potential; impermeability to microorganisms and dust particles; pH control; gas composition and humidity of the environment around the wound. Therefore, one of the major requirements for advanced wound coverings (dressings) implies their maximum action polyvalence [9–11].

There are over 470 samples of various dressings and wound coverings available now globally and coming in various pharmaceutical forms, whereas the basis for their manufacture includes polymers, collagen, gelatin, cellulose, pectin and many other substances, including various combinations of these materials that feature the respective properties and offer enough pathogenetic grounds to use them to treat wounds [12–14]. This means that one of the promising ways to improve the quality of the treatment offered to patients with infected and purulent wounds implies a scientific and experimental search for new dressings or modifying the already available ones.

Taking into account the above-described requirements for modern wound coatings, one of the most important properties that such materials should possess is biodegradation. From this stance, we believe chitosan — a derivative of the natural polysaccharide chitin — is to deserve special attention [15–18].

The increased interest taken in it seen as a basis for the production of wound coatings can be explained by its capacity to change its physical and chemical properties depending on changes in the molecular weight and three-dimensional structure. At the same time, the form factor of the product can change from a hydrogel to a frame structure with different degrees of density, swelling and moisture absorption. The specific features inherent in this polymer and the materials made on its basis explain the potential for its use. [19].

Such features include minimal side effects, biocompatibility, high healing potential, moisture- and air-permeability, high porosity, mechanical stability combined with plasticity, and a predictable bioresorption period within the body. Chitosan possesses hemostatic, bacteriostatic, and fungistatic properties. There have also been immunomodulating and antitumor effects identified, as well as lack of immunoreactivity has been proven along with complete elimination out of the body and biostimulation of regeneration [20, 21]. The study has also shown that chitosan films can inhibit significantly the growth of microflora (Staphylococcus, Proteus, Pseudomonas aeruginosa), at the same time accelerating the healing of burns. The universal mechanism, which promotes selective binding of chitosan to sugar receptors on the cell membrane, produces a bacteriostatic effect, which, in turn, stops the infectious process involving the most significant microorganisms [22, 23].

In view of all the properties mentioned above, which are featured by the proposed chitosan-based coatings, we can talk about the holding a research project aiming at developing dressings with a polyvalent action range.

Aim of Study:

To expand the range of advanced multifunctional chitosan-based wound coatings with significant antimicrobial and healing properties; to study in vivo the specific features of the wound process involving the treatment of purulent soft tissue wounds of various origins.

METHODS

Study Design

A multi-stage randomized controlled experimental study involved 225 conventional male rats, weighing 250–300 g each — at Stage 1; 135 individuals of linear Wistar male rats, weighing 250–300 g each — at Stage 2, and 27 male rabbits (Soviet Chinchilla breed), weighing 2,900 (±150) g each — at Stage3 of the study.

To create a model of purulent soft tissue wound in experimental animals, we employed our own technique (Patent for invention RU # 2703709 of August 23, 2018). This comes down to developing a wound featuring specified parameters through inserting a rounded polymer implant into the soft tissues for 6-7 days. Wound infection in animals was modeled through the introduction of Staphylococcus aureus (S. aureus 209P (ATCC6538P)) on foam balls for soft application of liquid and paste-type medicines Pele Tim # 3 (VOCO, Germany). To create infecting, a suspension of an 18–20-hour microorganism culture in a saline solution was used (concentration — 10^5 microbial bodies per 1 ml). 0.1 ml of bacterial suspension was injected in each ball with a sterile disposable syringe (1 ml) (Beijing Fornurse Medical Equipment Co., Ltd. Ltd, China).

The infecting dosage was 105 CFU of S. aureus 209P per animal. The implant was inserted through a layer-by-layer dissection of soft tissues down to the required depth. Further on, the wound was sutured in layers for the above-mentioned period. The implant was removed surgically, and the tested material was inserted into the resulting cavity. In the control group, the animals did not have the material sample injected into the wound. The tissue defect above the experimental cavity was layer-by-layer sutured with apposition interrupted sutures. The surface area (S) and volume (V) of the cavity were calculated using the following formulas: $S=4\pi r^2$, $V=4/3\pi r^3$, where r is the radius of the implant, $\pi = 3.14$.

Throughout the study, the skin sutures were treated with an antiseptic (the trade name of the antiseptic is not mentioned here thus aiming to avoid a conflict of interest).

Experimental samples of multifunctional chitosan-based wound coatings were developed and synthesized on the basis of the National Research

Center "Kurchatov Institute" (Table 1). The specific issue about the materials was the density and the pore orientation — isotropic or vertical.

Major study outcomes and registration methods

The main outcome of the study was the identification of the most appropriate sample in terms of its parameters in the experimental groups, as well as the observed differences in the wound process dynamics between the control group and the experimental one.

An additional expected outcome was compliance with the data obtained through non-invasive ultrasound imaging of the wound defect area, in order to study changes in the structure of the tested material samples undergoing biodegradation, as well as the dynamics of changes affecting tissues in the area of the experimental wound.

The study involved analyzing the wound process parameters (reduction of the wound volume, healing rate, sample biodegradation rate, complications, histomorphological composition of the wound) while using various samples of the designed wound coating. Visual assessment of the wound process course included keeping track of the timing of reduction of edema, hyperemia of the tissues around the wounds, as well as any discharge from the wound.

The tissues were subjected to histomorphological assessment following the generally accepted algorithm. The explanted tissues were fixed for 3–5 days in a 10 % neutral formalin solution (Histolab, Sweden), washed in running water for 60 minutes. The materials were processed subject to the standard procedure employing an automatic method on a Leica TP1020 histoprocessor (Germany). The production of paraffin blocks with samples of the studied materials was carried out using a modular installation Leica EG1150H (Germany). A rotary microtome Leica RM2235 (Germany) was used for slicing the preparations, while the obtained sections (thickness — 5 microns) were stained with hematoxylin and eosin according to the standard method. The micro-preparations were studied on an Olympus XH41 microscope (Japan). The morphological evaluation of the created materials structure was done in the Versa 3D SEM/FIB DualBeam scanning election microscope (FEI, USA) with various accelerating voltages, equipped with a Gaseous Second-

Table 1. *Technological features of chitosan samples used through the experiment*

Sample	Porosity	Wall thickness range	Pore size range	Elasticity modulus	Deformation in compression	Offset yield strength
	[%]	[nm]	[μM]	[MPa]	[%]	[kPa]
#1	98	350-1000	20-45	0.749	44.32	29.70
#2	98	600-1200	70-200	0.369	50.14	24.15

Table 1 offers a view at the major parameters featured by the samples under study.

All experimental surgical interventions performed on laboratory animals were done in aseptic conditions and under general anesthesia. A combination of drugs (atropine sulfate, prednisone, Sedamidine, Telazol) was used as the anesthetic.

To reduce the general anesthesia time, upon completion of the respective manipulations, the animals were injected with Antimedin. To relieve the pain syndrome within the postoperative period (Day 1), the animals were injected with Flexoprofen (intramuscular).

Inclusion criteria

For the purposes of the study (through all of its stages), male rats, as well as male rabbits were used with no external signs of diseases and anatomical disorders, that were quarantined in the retainer unit of the academic & production department of the Kuban State Medical University. The study involved animals that developed purulent wounds with standard signs of purulent inflammation during the simulation period.

Environment of study

Through the entire study, including postoperative periods, the animals were monitored with free access to water and food, in accordance with Russian State Regulatory Standards 33044-2014 "Principles of Good Laboratory Practice". The study was carried out on the premises of the training and production department of the Kuban State Medical University (Krasnodar, Russia).

Duration of study

Each stage of the study went on for 28 days, taking into account the 7 days required for the wound cavity development. Measurements, diagnostics, and sampling of the material for histomorphological examination at all the stages were done on Days 7, 14, and 21.

ary Electron Detector, a Peltier-cooled table and an option of using the ambient mode.

Ultrasound examination of the tissues was carried out with a Mindray M7 ultrasound scanner, using a high-frequency linear ultrasound sensor L 12-4s (China), with an operating frequency of 6–10 MHz — on the day the surgery was performed and then two days later, until the animal was removed from the experiment, in the following modes: in the color Doppler mapping mode using a pulse-wave Doppler as well as in the constant-wave Doppler mode.

Statistical analysis

At Stage 1, 2 samples of wound covering were examined in different modifications, for which 4 experimental and 1 control groups were formed, 45 animals (conventional male rats) in each group. Within the control term time, 15 animals within each group were removed from the experiment.

Stage 2 involved studying 2 types of wound coating samples of (# 1 and # 2) based on the criteria of porosity, mechanical strength and biodegradation terms. The properties of the samples were studied in 2 experimental and one control groups, each including 45 animals (linear male Wistar rats). Within the control period, 15 animals of each group were removed from the experiment.

At the final stage, male rabbits were used, which were broken into 2 experimental and 1 control groups, 9 animals in each. In each control period, similar to the previous ones, 3 animals of each group were removed from the experiment.

The statistical processing of the study results was done on a personal computer in the Windows 10 operating system using the STATISTICA 6.1 (StatSoft, Inc., USA) and Excel (Microsoft Office 2010) software. The hypothesis of the cumulative distribution normality in the samples was tested relying on the Shapiro-Wilk and Kolmogorov-Smirnov criteria. The statistical significance level was set at $p<0.05$. The differences between the quantitative parameters with a normal distribution were evaluated through the Student's t-test, whereas independent samples were evaluated relying on the nonparametric Mann-Whitney test. The differences in all cases were considered statistically significant at $p<0.05$. The significance level of the relationship between the two qualitative variables was tested through Pearson's Chi-square (χ^2) test.

RESULTS

Major results of study

Preliminary screening studies carried out at Stage 1 of this experiment revealed 2 most promising types of wound coatings made of modified chitosan, which featured fundamental differences, both physical and chemical. This allows differential change in the tactics of purulent wound local treatment, depending on the specific task.

Stage 2 of the experiment implied studying the samples of wound coatings selected through Stage 1 following the criteria of porosity, mechanical strength and biodegradation time. The properties of the samples were studied in conditions of a purulent wound. The result of the study revealed certain advantages demonstrated by sample # 1. Its biodegradation process was uniform; the distribution in the wound cavity was equal, which contributed to a smooth therapeutic effect in all parts of the wound cavity.

The assessment of the biodegradation degree of the samples studied at Stage 2 in % ratio to the initial volume revealed a statistically significant difference in biodegradation between samples # 1 and # 2. Sample # 2 underwent biodegradation 7 times as fast ($p<0.01$) and by Day 9 was basically undetectable within the operation area (Fig. 1).

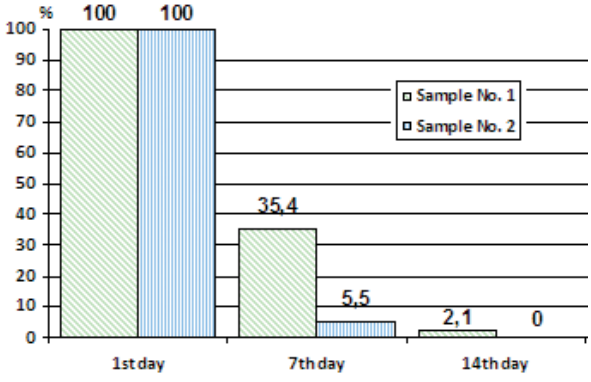
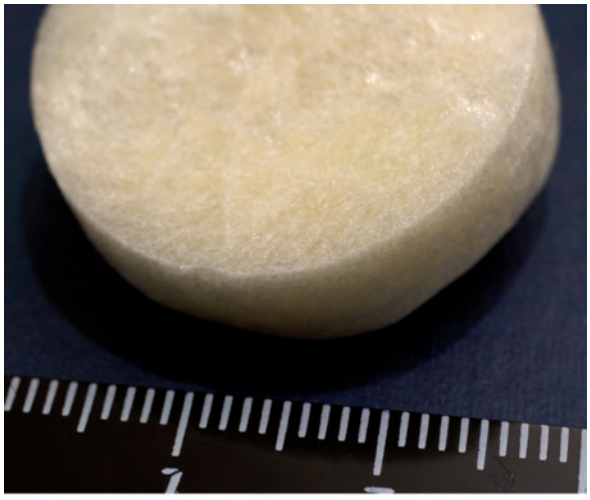


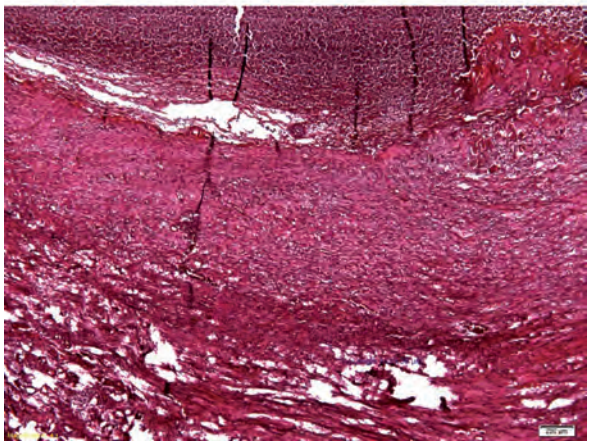
Fig. 1. *Residual wound coverage volume (% of original) in the target time frame – p<0,01.*

The histological representation, following the introduction of a test material sample, is a developed abscess in the muscle tissue, where the walls consist of 2 layers — a pyogenic membrane (up to 0.3 mm) and a developing connective tissue capsule (up to 1.5 mm). The abscess cavity contained a partially biodegraded sample distributed evenly. The muscle tissues surrounding the capsule featured an edema with an increased number of full-blood capillaries, with certain signs of stasis and lymph-macrophage infiltration (Fig. 2).

Stage 3 was carried out in order to study the chitosan-based multifunctional wound coating properties in larger purulent wounds affecting soft tissues.



a) Appearance of sample No. 3, with a magnification of x4



b) Fragments of sample no. 3; homogeneous biodegradation (7 days)

Fig. 2. *a) — Appearance and histomorphological picture of sample No. 3, with signs of homogeneous biodegradation on the 7th day after its introduction into the experimental wound b) — (1 — homogeneous biodegradation of the material; 2-pyogenic capsule).*

The area chosen to develop the wound cavity was the shoulder-scapular region, whereas the trapezius muscle tissue was chosen as the depth of the simulated cavity.

The polyvalent potential of using the developed samples is due to the experimentally proven properties featured by the selected samples: proper frame function (sufficient density), due bioadhesive capacity, biodegradability and biocompatibility.

All these properties identified in experimental samples were to be found in them in different proportions, which could be accounted for by the specifics of their production technology — the share chitosan in the base solution being the first one, as well as the molecular weight of the sample; features of the pore internal orientation and the ratio of the size and thickness of the walls separating them.

Given that, a programmable change in the initial parameters' ratio of the raw stuff and the use of various options for manufacturing chitosan samples, allow altering the finally obtained properties of the developed wound coatings as listed above.

Extra results of study

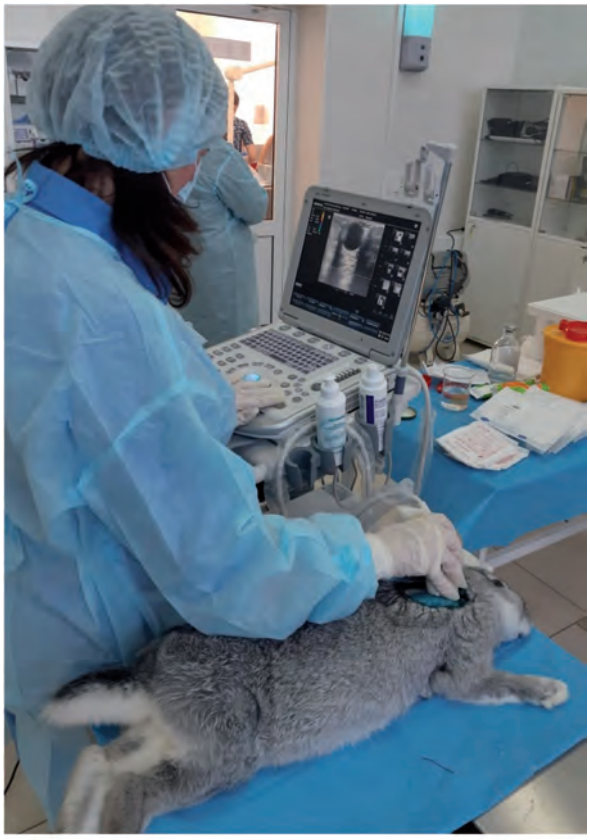
At Stage 3, the experiment was carried out relying on intravital ultrasound of the wound defect area (Fig. 3). The purpose of the ultrasound study was to investigate possible visualization of changes affecting the structure of the test material samples undergoing biodegradation, as well as the dynamics of tissue changes at the experimental wound.

The use of ultrasound to monitor the experimental wounds through the experiment, done in order to ensure a more detailed study of the wound cavity and the surrounding tissues status could be explained by a number of reasons. At the stage of developing the wound cavity, the ultrasound method allowed visualizing the implant inserted into the soft tissues as well as the blood flow in the surrounding tissues, which helped assess the location of the implant in relation to the surrounding anatomical formations, and was of great importance for standardizing the process of the wound cavity development. In view of the dynamics, the ultrasound study allowed identifying the degree of the wound cavity capsule development, any wound discharge inside the cavity, as well as the blood flow status at the surgical intervention area.

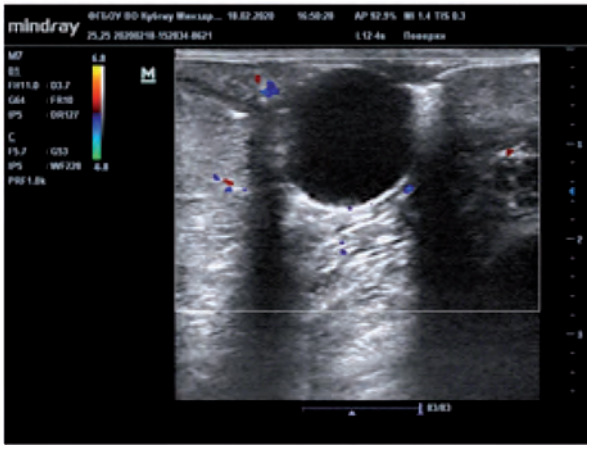
At the further stages of the experiment, the method of ultrasound examination of the experimental wound area containing a coating sample allowed not only visualizing biodegradation of the tested material, yet also identifying the major ultrasound semiotics of soft tissue damage – inflammatory infiltrate at different stages of its progress, muscle fiber imbibition, the appearance of a purulent substrate and signs pointing at the beginning reparation.

The verification of the data obtained through the ultrasound examination was done by studying and comparing the biopsy material obtained when removing the animals from the experiment within the above-described control periods. This method helped us arrive at non-invasive assessment of the wound process status when using the selected samples of wound coatings, as well as it helped visualize the samples of the studied material, which were undergoing biodegradation (Fig. 4).

Following the introduction of chitosan samples into the muscle layer, they were visualized as formations featuring clear and even contours and a homoge-



a) Conducting an ultrasound scan at the stage of introducing a polymer implant into soft tissues at stage 3 of the experiment



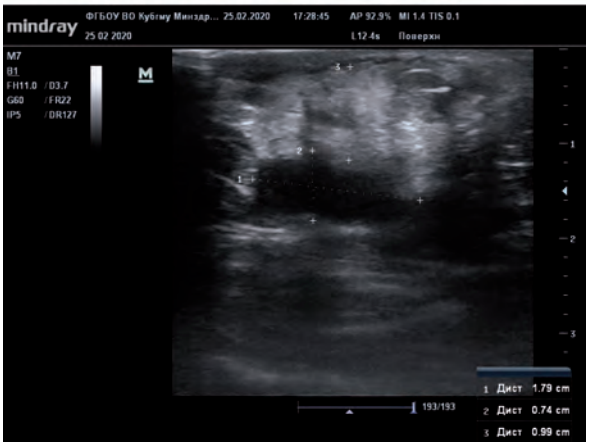
b) Assessment of the blood supply to the surrounding tissues (1) (2)

Fig. 3. *a) Ultrasound at the stage of introducing a polymer implant into soft tissues. b) — Assessment of blood supply of tissues (2) surrounding implant (1)*

neous structure with the effect of distal pseudo-reinforcement, the size being up to 20–20–20 mm.

Along the periphery of the implanted sample, there were vital tissues with clear ultrasound structure

a) Introduction of a sample of the test material (1) into the formed experimental wound



b) Ultrasonic location of the sample (1) of the test material in soft tissues

Fig. 4. *Comparison of a) visual and b) CD images of tissues and samples of the drug, when performing various stages of the study*

and echogenicity visualized. Vascular structures of muscle tissue were visualized as single blood flow loci revealing a spectrum of arterial and venous blood.

By Day 7 into the observation, the size of the wound coating samples regressed. What is more, sample # 2 had nearly completely biodegraded: the size of the wound cavity shrank down to 5–10% of its initial size. In the remaining wound cavity, a small amount of exudate (up to 0.25 ml) was to be observed. During that, the size of sample # 1, which was the denser one, as well as the size of the cavity it was placed in, shrank down to 30–35% of its initial size. The peripheral area of the remaining wound cavity featured denser hyperechogenic and homogeneous layers of muscle fibers of various thicknesses (2.5 mm to 12 mm) appearing there.

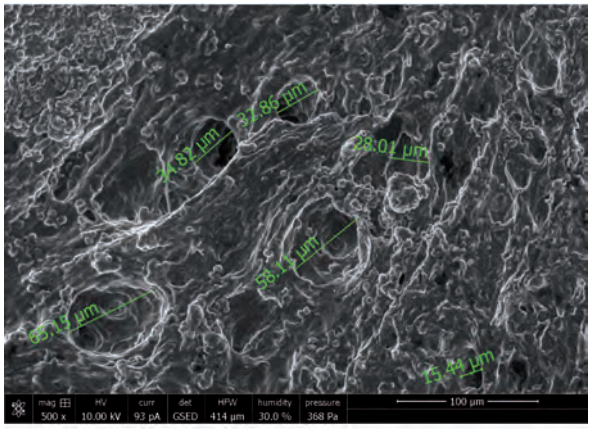
There was also an increase observed in the blood flow speed within the arterial and venous network – Vmax art — 25–30 cm/s; Vmax veins – 15–17 cm/s.

The study conducted on Day 14 into the experiment revealed that infiltrative changes affecting the muscle tissue surrounding the experimental wound cavity featured different stages of inflammation. There were signs of dense and loose infiltrate (layer thickness ranging from 1.7 mm to 9.8 mm) visualized, as well as the muscle fiber imbibition, with an increase in its volume (from 4.4 mm to 12.8 mm). The cavity of the wound defect, where the denser sample # 1 was implemented, decreased to 1.2 x 4.3 x 1.7 mm (H x W x D), with some exudate identified, which manifested itself as anechogenic strips on the periphery of the sample residues (up to 0.5 ml).

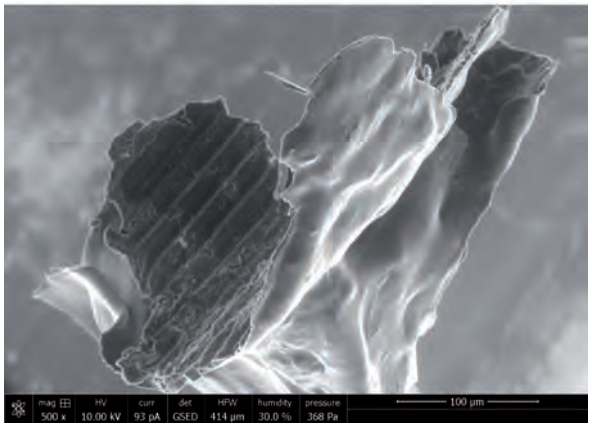
Upon introducing sample # 2, the residual effects in the experimental wound were visualized as loose-fibrous (hyperechogenic) formations with a diameter of up to 2.5 mm, which featured signs of infiltrative changes in the surrounding tissues. The maximum speed of the venous blood flow in the infiltrate zone was Vmax veins — 5 cm/s, which almost matched the initial value.

There was also a clear visualization of the boundaries separating the muscle-aponeurotic layers in the respective area, with a differentiation of a striated muscle tissue pattern.

To further study the tested wound coating sample biodegradation, we relied on the scanning electron microscopy method. On Day 7 into the experiment, samples # 1 were obtained. The result of the study confirmed the theoretical calculation of the step-by-step sample biodegradation with the capillary (sorption) properties preserved (Fig. 5).



a) Cellular structure preserved in the sample



b) Internal — with preserved structure (1) and external — partially biodegraded (2) surfaces of the test material in soft tissues

***Fig. 5.** Investigation of the biodegradation process by scanning electron microscopy*

Adverse events

No adverse events were observed through the experimental study.

DISCUSSION

The developed wound coatings featured proper framework functions, as well as due bioadhesive capacity, biodegradability and biocompatibility.

The study results obtained through investigating the properties of the developed chitosan-based wound coatings correlate with the literature data in terms of comparing properties like bioadhesive capacity and biodegradability [21]. The experimental data appear comparable with the results other researchers obtained — there was confirmation obtained for the calculated qualities of the impact that the respective samples had on the surrounding tissues, in view of the specifics of the wound defect occurrence and specific wound healing processes, as well as there was potential identified for ensuring a proper microclimate: controlled pH, humidity; thermal insulation of the wound; creating a barrier against microorganisms; ensuring due sorption activity of the material; atraumatic capacity in terms of granulations [15–17].

A comparison of the developed wound coatings revealed that various synthesis technologies allowed controlling their physical and chemical properties as well as possible use of these coatings within various treatment schemes not only when dealing with issues of infectious origin, yet also when treating other soft tissue lesions.

CONCLUSION

The chitosan-based wound coatings studied through a multi-stage experiment offered proof to the properties predicted while developing the materials, namely, a stable adhesive capacity to biological tissues and the framework function.

Sample # 1 featured moderate strength, elasticity, as well as a denser structure and, respectively, a lower level of biodegradation (bioresorption on Day 14), which determined its capacity to maintain the desired shape and the initial dimensions for a long time. This, in turn, allowed the sample to effectively perform the framework functions and ensure proper drainage of the pathology focus. The mentioned features revealed that the sample could offer a promising choice when dealing with purulent wounds.

Sample # 2 was of lower strength, elasticity, density, as well as a higher adhesion degree (compared to sample # 1), with complete biodegradation achieved on Day 7. In view of the test sample features, it can prove of the best use in cases with no purulent inflammation and with granulating wounds. Besides, sample # 2 revealed a capacity to transform into a gel with a highly ordered internal structure of the micellar type, which allowed introducing another function – the use as a carrier matrix for the drugs introduced into its structure. This condition will allow expanding the indications for use.

The parallel ultrasound examination technique employed to study experimental wound areas produced an expected positive effect. The match between the data obtained during non-invasive visualization of the analyzed samples and the surrounding tissues, with the data obtained through histomorphological studies allowed reducing both the number of invasive procedures and the number of animals to be involved in the study process.

ETHICS PRINCIPLES COMPLIANCE

The study described above was granted approval at the meeting of the Independent Ethics Committee, the Kuban State Medical University (Protocol # 63 of May 21, 2018) and was carried out within the period of 2019-2021.

FUNDING

The authors herewith claim no sponsorship used for the study.

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PREVENTION OF POST-ERCP PANCREATITIS IN HIGH-RISK PATIENTS

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ABSTRACT — Aim. To study the efficacy of thoracic epidural analgesia (TEA) for the prevention of post-ERCP pancreatitis in high-risk patients.
Materials and methods. A parallel, blinded, randomized study. The first (TEA group) group included patients (n = 98) in whom thoracic epidural analgesia was used during endoscopic transpapillary interventions (ETI), the second (OAI group) group included patients (n = 97) in whom opioid analgesics and indomethacin (per rectum).
Results. The study showed that acute pancreatitis was diagnosed significantly less frequently in patients with the TEA group than in patients with the OAI group (p = 0.0135). If in the TEA group post-ERCP pancreatitis (PEP) was verified in 3.1% (3/98) patients, in the OAI group — in 12.4% (12/97) patients.
When TEA was used in high risk patients of developing post-ERCP pancreatitis, its incidence decreased from 23.3% (10/43) to 4.4% (2/46) observations (p = 0.0095).
Conclusion. The use of TEA is an effective and justified method of prevention in patients at high risk of developing post-ERCP pancreatitis. In patients with a low risk of developing this complication, the use of TEA is inappropriate due to the invasiveness of the method.

KEYWORDS — therapeutic ERCP (endoscopic retrograde cholangiopancreatography), prevention of post-ERCP pancreatitis, thoracic epidural analgesia.

INTRODUCTION

The use of endoscopic transpapillary interventions (ETI) to correct biliary hypertension syndrome has significantly improved the results of treatment in patients with pathology of the duodenopancreatobiliary zone.

At the same time, ETI are complex interventions with unpredictable consequences. During the perioperative period, severe and sometimes fatal complications may occur, such as post-ERCP pancreatitis (PEP), bleeding from the papillotomy area, and retroduodenal perforation [1, 2, 3, 4].

According to many researchers, the incidence of post-ERCP pancreatitis is within 1–40% of observations and depends on many factors: the nature of the disease, the type of endoscopic intervention, the patient's age [2]. Such factors as young age, sphincter of Oddi dysfunction, the use of balloon dilatation for ETI, the absence of jaundice increase the risk of its development [3, 5, 6, 10]. To date, mortality in post-ERCP pancreatitis reaches 3–10%, and with developing infected pancreatic necrosis can reach 25–80% of cases [1, 2, 7, 8, 11].

In recent years, there have been positive responses from the research community on the effectiveness of non-steroidal anti-inflammatory drugs (diclofenac and indomethacin) and thoracic epidural analgesia for the prevention of PEP in ETI [1, 12]. Thus, we decided to conduct a comparative analysis to test the effectiveness of these two methods of preventing post-ERCP pancreatitis in high-risk patients.

MATERIALS AND METHODS

Our parallel, unblinded, randomized study was approved by Volgograd Regional Ethics Committee. All participants signed an informed consent.

223 ETI were conducted from January 2019 to December 2020. All patients were hospitalized. In 13 (4.9%) patients, endoscopic intervention was performed for diagnostic purposes (excluded from the study). In 9 out of 223 (4.0%) patients, signs of acute pancreatitis were diagnosed before the intervention (excluded from the study). One patient refused to participate in the study. A total of 200 patients were included in the study.

Prior to the formation of the database, the study design, inclusion and exclusion criteria were determined.

Inclusion criteria:

1. Completed therapeutic ETI;
2. Prior to ETI, the patient had no clinical signs of acute pancreatitis.

Exclusion criterion: a complication (retroduodenal perforation, massive bleeding, detachment of the Dormia basket) was diagnosed during ETI and required an urgent surgical intervention.

All subjects were divided into two groups (100 patients each). The first (TEA group) group included patients in whom TEA was used during ETI, the patients of the second (OAI group) group received a narcotic analgesic and indomethacin.

Puncture and catheterization of the epidural space was performed according to the standard tech-

nique at the level of the VII–VIII thoracic vertebrae. 20 minutes before ETI, a solution of ropivacaine 0.5% – 10 ml was injected into the epidural space.

In the TEA group, two patients were excluded from the study due to unsuccessful attempts to insert a catheter into epidural space (1 case) and retroduodenal perforation (1 case). In the OAI-group, three patients were excluded from the study: due to massive bleeding (1 case), retroduodenal perforation (1 case), and retention of the Dormia basket (1 case), which required surgical interventions.

As a result, the results of treatment of 195 patients (98 patients of the TEA group and 97 patients of the NAI group) were analyzed.

The studied indicators (demographic data, the nature of the disease, the results of laboratory or instrumental research, outcomes, etc.) were entered into the database within 10 days after the intervention.

Statistical analysis of the data was performed using nonparametric tests (OR and Fisher's exact test). The groups were divided into subgroups taking into account age, sex, nature of the disease, severity of concomitant pathology, types of ETI. For each of the subgroups, the odds ratio (OR) was calculated with a 95% confidence interval (95% CI). A statistically significant difference between the study groups (subgroups) was considered $p \leq 0.05$ (an indicator of statistically significant differences) or when the 95% confidence interval (CI) did not include 1. Statistical processing of the data was carried out using a set of statistical programs Statistica 10.0 (StatSoft Inc., USA).

RESULTS AND DISCUSSION

The demographic data of the patients are presented in Table 1. In the TEA-group, the average age of the patients was 61.4 ± 1.3 years, in the OAI-group — 60.9 ± 1.2 years. There were no statistically significant differences in the number of women and patients of young age (under 60 years) and more advanced age (over 60 years) (OR 0.94 [95% CI, 0.53–1.66]) and (OR 1.06 [95% CI, 0.60–1.88], respectively).

The study groups did not differ in other indicators either. In the TEA group and the OAI group, there was a commensurate number of patients with tumors of the hepatopancreatobiliary zone (33.7% [33/98] versus 33.0% [32/97] cases, OR 1.03) and with jaundice (38.8% [38/98] versus 38.1 % [37/97] cases, OR 1.03); with choledocholithiasis (36.7% [36/98] versus 38.1% [37/97] cases, OR 0.94) and with sphincter of Oddi dysfunction (SOD) (12.2% [12/98] versus 11.3% [11/97] observations, OR 1.09).

Concomitant diseases were detected in 58.2% (57/98) of patients in the TEA group and in 61.9% (60/97) of patients in the OAI group (OR 0.85 [95% CI, 0.48–1.53]). The study groups also did not differ in the number of patients with severe comorbidity (ASA IV) (OR 1.13 [95% CI, 0.41–3.08]). The majority of patients in this category were diagnosed with diseases of the cardiovascular system (84.2% [48/57] of patients with the TEA group and 85.0% [51/60] of patients with the OAI group).

The study groups also did not differ on other variables. Based on the above, the study groups represented patients with similar baseline parameters, and this could not affect the reliability of the results.

Depending on the nature of the disease, various types of endoscopic transpapillary interventions were used. The study groups did not differ in this indicator either. In the TEA group and the OAI group, endoscopic papillosphincterotomy was equally often used (in 82.7% versus 81.4% of cases (OR 1.09 [95% CI, 0.52–2.27])) and balloon dilation (in 18.4% versus 20.6% of cases (OR 0.87 [95% CI, 0.42–1.77])), biliary-stone extraction (in 51% versus 53.6% cases (OR 0.90 [95% CI, 0.51–1.59])) and lithotripsy (in 9.2% versus 10.3% of cases (OR 0.88 [95% CI, 0.34–2.29])), installation of biliary stent (31.6% versus 28.9% of cases (OR 1.14 [95% CI, 0.39–3.32])) and installation of pancreatic stent (19.4% versus 22.7% of cases (OR 0.82 [95% CI, 0.41–1.65])).

During this study, patients in the study groups were diagnosed with 15 (7.7% [15/195]) cases of PEP development. In 80.0% (12/15) of observations, this complication was registered in patients of the OAI-group (Table 2). The incidence of PEP in the TEA group was 3.1% (3/98), and in the OAI group — 12.4% (12/97) of observations, which is a statistically significant difference between the study groups ($p = 0.0135$).

The vast majority of cases of PEP in patients of the TEA group and OAI group were mild (66.7% [2/3] and 58.3% [7/12] observations, respectively) and their clinical manifestations could be stopped within 3 days. One of three patients (33.3%) of the TEA group and 4 of 12 (33.3%) patients of the NAI-group needed to continue anti-pancreatic therapy for up to 7 days. Taking into account the clinical picture, the data of laboratory and instrumental studies, in the NAI-group, pancreatonecrosis was verified in 1 (1.0%) patient. Symptoms of pancreatic necrosis in this patient developed rapidly and were characterized by total damage to the pancreas, complicated by the development of multiple organ failure, which led to death.

The incidence of post-ERCP pancreatitis in study subgroups different in gender, age, disease nature, severity of concomitant pathology and types of intervention is shown in Table 3.

A statistically significant decrease in the incidence of PEP with the use of TEA was found in patients

Table 1. *Selected subject and procedural characteristics of patients*

Variable	Total, n (%)		OR (95% CI)
	TEA group (N=98)	OAI group (N=97)	
Age			
18-40 y	9 (9.2)	8 (8.2)	1.13 (0.41-3.08)
41-60 y	38 (38.8)	40 (41.2)	0.90 (0.51-1.61)
61-80 y	39 (39.8)	36 (37.1)	1.12 (0.62-2.00)
>80 y	12 (12.2)	13 (13.5)	0.90 (0.39-2.11)
Gender			
Woman	59 (60.2)	61 (62.9)	0.89 (0.50-1.60)
Context			
Jaundice	38 (38.8)	37 (38.1)	1.03 (0.57-1.84)
Common bile duct stones	36 (36.7)	37 (38.1)	0.94 (0.52-1.69)
SOD	12 (12.2)	11 (11.3)	1.09 (0.45-2.63)
Common bile duct stones and SOD	14 (14.3)	15 (15.5)	0.91 (0.41-2.02)
Tumor	33 (33.7)	32 (33.0)	1.03 (0.56-1.88)
Calculous pancreatitis	3 (3.1)	2 (2.1)	1.50 (0.24-9.37)
ASA grade			
IV	9 (9.2)	8 (8.2)	1.13 (0.41-3.08)
III	21 (21.4)	22 (22.7)	0.93 (0.47-1.84)
I and II	68 (69.4)	67 (69.1)	1.01 (0.55-1.88)
Procedural			
Biliary sphincterotomy	81 (82.7)	79 (81.4)	1.09 (0.52-2.27)
Balloon dilation	18 (18.4)	20 (20.6)	0.87 (0.42-1.77)
Biliary-stone extraction	50 (51.0)	52 (53.6)	0.90 (0.51-1.59)
Installation of biliary stent	31 (31.6)	28 (28.9)	1.14 (0.61-2.12)
Lithotripsy	9 (9.2)	10 (10.3)	0.88 (0.34-2.29)
Installation of pancreatic stent	19 (19.4)	22 (22.7)	0.82 (0.41-1.65)
Naso-biliary drainage	8 (8.2)	7 (7.2)	1.14 (0.39-3.32)

Note. **P < 0.05, statistically significant; ASA — American Society of Anaesthesiologists; SOD — sphincter of Oddi dysfunction*

Table 2. *The incidence of post-ERCP pancreatitis in patients of the study groups*

Post-ERCP pancreatitis	TEA group (N=98)	OAI group (N=97)	P, Fisher
Mild, n(%)	2 (2.1)	7 (7.2)	0.0823
Moderate, n(%)	1 (1.0)	4 (4.2)	0.1811
Severe, n(%)	0 (0.0)	1 (1.0)	0.4974
Total, n(%)	3 (3.1)	12 (12.4)	0.0135*

under 60 years of age (OR 0.18 [95% CI, 0.04–0.93]) and with the use of endoscopic papillosphincterotomy (OR 0.17 [95% CI, 0.04–0.85]).

A significant (but statistically insignificant) decrease in the incidence of PEP with the use of TEA was found in patients of all study subgroups: in women (OR 0.31 [95% CI, 0.08–1.24]), in patients with jaundice (OR 0.22 [95% CI, 0.02–2.20]) and without it (OR 0.22 [95% CI, 0.04–1.14]), in patients with choledocholithiasis and SOD (OR 0.21 [95% CI, 0.02–2.29]) and in patients with isolated choledocholithiasis (OR 0.24 [95% CI, 0.02–2.32]), after balloon

Table 3. *The incidence of post-ERCP pancreatitis in patients of the study subgroups*

Variable	Post-ERCP pancreatitis n (%)		OR (95% CI)
	TEA group (N=98)	OAI group (N=97)	
Age			
18-40 y	1/9 (11.1)	2/8 (25.0)	0.38 (0.03-5.46)
41-60 y	1/38 (2.6)	6/40 (15.0)	0.15 (0.02-1.40)
61-80 y	1/39 (2.6)	3/36 (8.3)	0.29 (0.03-3.06)
>80 y	0/12 (0.0)	1/13 (7.7)	---
Gender			
Woman	3/59 (5.1)	9/61 (14.8)	0.31 (0.08-1.24)
Men	0/39 (0.0)	3/36 (8.3)	---
Context			
Jaundice	1/38 (2.6)	4/37 (10.8)	0.22 (0.02-2.20)
Common bile duct stones	1/36 (2.8)	4/37 (10.8)	0.24 (0.02-2.32)
SOD	0/12 (0.0)	1/11 (9.1)	---
Common bile duct stones and SOD	1/14 (7.1)	4/15 (26.7)	0.21 (0.02-2.29)
Tumor	1/33 (3.0)	3/32 (9.4)	0.30 (0.03-3.22)
Calculous pancreatitis	0/3 (0.0)	0/2 (0.0)	---
ASA grade			
IV	0/9 (0.0)	1/8 (12.5)	---
III	1/21 (4.8)	3/22 (13.6)	0.30 (0.03-3.31)
I and II	2/68 (2.9)	8/67 (11.9)	0.22 (0.04-1.13)
Procedural			
Biliary sphincterotomy	2/81 (2.5)	10/79 (12.7)	0.17 (0.04-0.85)*
Balloon dilation	1/18 (5.6)	5/20 (25.0)	0.18 (0.02-1.77)
Biliary-stone extraction	2/50 (4.0)	8/52 (15.4)	0.23 (0.04-1.18)
Installation of biliary stent	1/31 (3.2)	4/28 (14.3)	0.20 (0.02-2.00)
Lithotripsy	1/9 (11.1)	3/10 (30.0)	0.29 (0.02-3.67)
Installation of pancreatic stent	0/19 (0.0)	2/22 (9.1)	---
Naso-biliary drainage	1/8 (12.5)	2/7 (28.6)	0.36 (0.02-5.40)
Total, n(%)	3/98 (3.1)	12/97 (12.4)	0.22 (0.06-0.83)*

Table 4. *Frequency of post-ERCP pancreatitis in high-risk patients*

Post-ERCP pancreatitis	High risk patients		P, Fisher
	TEA group (N=46)	OAI group (N=43)	
Mild, n(%)	1 (2.2)	6 (14.0)	0.0454*
Moderate, n(%)	1 (2.2)	3 (7.0)	0.2830
Severe, n(%)	0 (0.0)	1 (2.3)	0.4831
Total, n(%)	2 (4.4)	10 (23.3)	0.0095*

dilatation (OR 0.18 [95% CI, 0.02–1.77]), biliary-stone extraction (OR 0.23 [95% CI, 0.04–1.18]) and naso-biliary drainage (OR 0.36 [95% CI, 0.02–5.40]), etc.

In some subgroups, patients from the TEA group did not have a single case of post-ERCP pancreatitis, namely, in patients over 80 years of age, in men, in patients with SOD, with calculous pancreatitis, with severe concomitant pathology (ASA IV), after installation of pancreatic stent. There were no lethal outcomes in patients of the TEA group.

More often, the early postoperative period was accompanied by the development of AKI in women (in 10.0% [12/120] cases), in young (up to 60 years old) patients (in 10.5% [10/95] cases), and in patients with choledocholithiasis and SOD (in 17.2% [5/29] observations). According to our data, these factors increased the risk of post-ERCP pancreatitis, and the subjects, who combined two or more risk factors, were attributed to patients with a high risk of PEP [1]. In the TEA group, 46.9% (46/98) were identified, and in the OAI group — 44.3% (43/97) of patients in this category. Table 4 presents data on the incidence of post-ERCP pancreatitis in patients at high risk of developing this complication.

As shown in the table, when TEA was used in patients with a high risk of PEP, its incidence decreased from 23.3% (10/43) to 4.4% (2/46) observations ($p = 0.0095$).

In patients with a low risk of developing this complication, the use of both TEA and indomethacin suppositories, as methods of preventing PEP, has shown commensurate efficacy. There was no statistically significant difference in these study subgroups (3.7% (2/54) observations in the NAI subgroup versus 1.9% (1/52) cases in the TEA subgroup ($p = 0.5143$)).

Throughout the entire period of the use of therapeutic ETI we havecontinued the search for methods of preventing the development of PEP. A lot of studies were carried out to study the preventive effect of the use of various groups of medicines (nitrates, sandostatin, heparins, etc.) [4, 6, 7, 11]. For this purpose, various endoscopic tactics of endoscopic interventions were proposed for use [1, 9, 10]. But despite this, development of post-ERCP pancreatitis has remained the main problem of therapeutic ETI.

As a result of our study, reliable data were obtained on the effectiveness of of TEA for preventing the development of PEP in ETI. A decrease in the incidence of post-ERCP pancreatitis from 12.4% to 3.1% was shown. In the TEA group, PEP was mild to moderate in all cases. The use of TEA helps prevent the development of pancreonecrosis and poor outcomes in patients, which significantly increases the safety of ETI in patients with a high risk of PEP and severe comorbidity. An important fact is that the use of TEA, as noted by endoscopists, creates more comfortable conditions for their work.

All patients were inpatient until the end of the study, so there was no data loss. The indicators were entered into the database in real time.

Of course, this method of prevention has its drawbacks. We refer to the main disadvantages: the need to involve a doctor who knows the methodology for conducting TEA; the invasiveness of the method (despite its safety); restriction in use in patients with coagulopathies (for example, in patients with hepatic insufficiency); the possibility of using only in hospitalized patients.

Of course, not all patients with therapeutic ETI need to use TEA. Obviously, in patients with a low risk of developing PEP, it seems sufficient to use a less invasive technique — indomethacin suppositories.

CONCLUSION

— The use of TEA is an effective and justified method of prevention in patients at high risk of developing PEP;

— In patients with a low risk of PEP, the use of TEA is inappropriate due to the invasiveness of the method.

In conclusion, we would like to note that this study does not question the effectiveness of other prevention methods recommended by different authors, but only complements them. Anyway, an individual approach to each patient is required.

Conflict of interests

The authors state that they have no conflict of interests.

Contributors

MIT and VVM collected, analysed, and interpreted data and made the figures. ASP did the literature review and collected data. AVE and YuIV collected data and made the figures. MIT and VVM interpreted and analyzed the data. MIT, ASP, AVE, YuIV and VVM prepared the manuscript for submission.

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CLINICAL SIGNIFICANCE OF LAMININ AND ELASTINE LEVELS IN CHILDREN WITH UNDIFFERENTIATED CONNECTIVE TISSUE DISEASE

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ABSTRACT — This research aims to study levels of laminin and elastine in 64 children with undifferentiated connective tissue disease (UCTD). All the children underwent clinical, laboratory and instrumental examination. It was found that changes in the levels of laminin and elastin are directly related to the severity of UCTD in children. Thus, clinical values of laminin and elastin levels can serve as additional criteria of UCTD severity. Using them, along with early detection of the phenotypic and visceral signs, helps prevent the development of severe forms of the disease.

KEYWORDS — undifferentiated connective tissue disease (UCTD), laminin, elastine, blood serum, severity criteria, children.

INTRODUCTION

The importance of detecting the undifferentiated connective tissue disease is challenged by its high incidence (up to 80%) and ambiguous interpretation of phenotypic and visceral signs. The diagnostic criteria for the degree of its severity are proposed by researchers from different countries [1, 2, 3, 4].

Evaluating the UCTD severity basing on phenotypic and visceral manifestations has often resulted in uncertainty and frustration, thus urging the search of additional biochemical criteria, including such important non-collagen proteins as elastine, laminin, etc. They control interrelation between the elastic core and microfibrils [5, 6, 7]. Considering their sensitivity and extensive diagnostic range, their detection may improve the evaluation of UCTD severity in children [8, 9]. All that justifies the urgency of the issue.

The aim of the research is to study clinical significance of laminin and elastine levels in children with UCTD.

CHARACTERISTIC OF THE CHILDREN; METHODS OF RESEARCH

64 children aged 3 to 10 years with UCTD have been under observation. 27 of them had mild UCTD severity (the 1st group), 19 possessed medium UCTD severity (2nd group), and 18 suffered from severe UCTD (3rd group). The control group consisted of 18 conditionally healthy children.

UCTD diagnosis was performed basing on life record data, phenotypic and visceral signs, excluding genetic syndromes. Over 6 external phenotype data was analyzed. Ultrasonic scans of visceral organs were carried out.

The levels of elastine and laminin in blood serum were examined with the help of sandwich-type polarization fluoroimmunoassay, using the kits produced by «Cloud-Clone Corp» (USA) to determine elastine quantifiable concentration (serial number HBE-337Hu No 4 DF58C0861) and laminin quantifiable concentration (serial number HBE082Hu No 7B578DDCCD). Data processing was performed with the methods of variable-based statistics (Statistika-10).

RESULTS AND INTERPRETATION

We defined 6–7 phenotypic signs in the first group. 65% of cases presented visceral signs of UCTD, including 13 cases of minor cardiac abnormalities (50%), 6 cases of mild pyelectasis (22,2%), 3 cases of nephroptosis (11,0%), and 3 cases of hypotension and cholecystis anomaly (11,0%). The levels of laminin and elastine in this group were close to normal range ($p1 > 0,05$; $p2 > 0,05$).

Within the second group of children, 95,8% had 10–12 moderate phenotypic signs, early evidence of which was observed in 5 children (26,3%). The visceral signs were presented in 14 (72,8%) of minor cardiac abnormalities, 5 cases of pyelectasis (26,3%), 3 cases of nephroptosis (15,8%), 3 cases of gallbladder volvulus (15,8%), and 5 cases with combination of signs (26,3%). The combination of phenotypic and visceral signs is 20% higher in the second group comparing with the first one.

The increase of elastine and laminin levels is statistically-valid (Table 1, $p_1 < 0,01$, $p_2 < 0,05$). The increase of proteins levels was associated with incidence of visceral signs combination ($k_1 = 0,72$; $k_2 = 0,61$).

As for the third group, the infantile onset of UCTD (in the first years of life) dysplastic indicants in 7 children (36,7%) was interpreted as *dysplastic march* with early incipience. All the children under observation showed 13–19 common phenotypic signs of mild and medium severity.

15 children under observation (83,4%) had combination of 15–17 phenotypic signs. Visceral signs were presented in 17 cases of minor cardiac abnormalities (95%), 7 cases of pyelectasis (38,8%), 4 cases of nephroptosis (22,2%), 3 cases of hydronephrosis (16,6%), 5 cases of gallbladder hypotension (26,7%), and 5 cases of gallbladder volvulus (26,7%). Comorbid syndromes (2,7) of renal and renocardial UCTD were present in 9 children (50,0%). Comorbidity of visceral signs in the third group was observed by 25% more often than in the second group, and was associated with increase in laminin and elastine levels correspondingly ($k_1 = 082$; $k_2 = 0,57$).

Statistically significant difference ($p_1 < 0,01$, $p_2 < 0,01$) was observed between the levels of laminin and elastine in groups with mild, medium and severe UCTD (see Table 1). Consequently, we detected changes in laminin and elastine levels in direct dependence on UCTD severity in children. The determined values of these proteins' levels may be used as additional criteria for evaluation of UCTD severity (Table 1).

Table 1. *The levels of laminin and elastine in groups with mild, medium and severe UCTD*

Data	Group			
	First (n=27)	Second (n=19)	Third (n=18)	Control (n=18)
laminin, (pg/ ml)	*, # 47,2 ± 6,3	*** 56,3 ± 7,8	*** 62,3 ± 8,2	24,20 ± 7,1
elastine (ng/ml)	*, # 9,2 ± 2,5	** 14,3 ± 1,7	*** 16,5± 1,4	7,2 ± 2, 2

Note: ** — reality in comparison of data of the 1st, 2nd and 3rd groups with control: (* >0,05, ** p<0,05, *** p<0,01; # — reality in comparison of data of the 1st and 2nd groups: # — p>0,05; ## — p<0,05*

CONCLUSION

Early evidence of visceral signs and their comorbidity are significant for UCTD diagnosis. It was found that changes in the levels of laminin and elastin are directly related to the severity of UCTD in children. Levels of laminin and elastine may serve as reasonable evaluation criteria of UCTD severity. The use of phenotypic, visceral and biochemical indicators of undifferentiated connective tissue disease may contribute to timely and exact diagnosis. Moreover, it may help to start the treatment sooner.

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FUNCTIONAL PROPERTIES OF PLATELET IN CHILDREN WITH IRRITABLE BOWEL DISEASE

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ABSTRACT — The aim of the study was to evaluate platelet activation and aggregation in children with irritable bowel disease (IBD), as well as the effect of hyperbaric oxygenation on these processes. Platelet activation and aggregation in artificial shear flow were studied in 120 patients of both sexes aged 6 to 17 years with IBD. In pediatric patients with CD and UC, a significant increase in the activation and aggregation of platelets was revealed under shear flow conditions. The use of hyperbaric oxygen therapy leads to a decrease in the studied processes. It was found that IBD in children is characterized by significant changes in the functional properties of platelets (activation and aggregation processes).

KEYWORDS — children, irritable bowel disease, platelet, hyperbaric oxygen therapy (HBOT).

INTRODUCTION

Inflammatory bowel diseases (IBD) are accompanied by quantitative, morphological and functional changes in platelets. The activated, hyperaggregable platelets are a key factor in enhancing the thrombogenic potential and intestinal microinfarctions. In addition, platelets initiate an inflammatory phenotype in endothelial cells and white blood cells and promote increased inflammation by secreting numerous biologically active substances [1]. Therefore, it becomes clear that it is necessary to study first of all the activation of platelets, which underlies the performance of their functions. To date, platelet activation is evaluated by measuring the concentration of circulating activation markers (P-selectin, CD40, and GP53) not only in adult patients, but also in children [2, 3]. Platelet activation is determined by the mechanisms that cause calcium to enter platelets and lead to conformational changes in the GP IIb/IIIa receptor complex on the cell surface and, consequently, the final common pathway — platelet aggregation.

The aim of the study was to evaluate the activation of platelets in an artificial shear flow (in a calcium-free environment) and their aggregation in children with IBD.

MATERIAL AND METHODS

The study used the blood samples of 120 patients of both sexes aged 6 to 17 years, suffering from IBD. The study was approved by the Local Ethics Committee of Privolzhsky Research Medical University. Informed consent to participate in the study was obtained from the parents of all children (or from the children themselves over the age of 15). The diagnosis of "IBD" was verified by the data of a comprehensive examination, which included clinical and laboratory data, as well as endoscopic examination of the intestinal mucosa with morphological analysis of biopsies. Blood sampling was performed at the time of hospitalization of patients in the acute stage and at the end of treatment, before discharge. The treatment was carried out with the use of 5-aminosalicylic acid derivatives, glucocorticosteroids, immunosuppressants, and genetically engineered biological therapy. The results of the studies were compared with similar indicators of 35 conditionally healthy children of both sexes of the same age, who made up the control group.

Spontaneous (flow-induced) platelet aggregation was studied under conditions of artificial shear flow on a rheoscope designed according to the principle [4] in the modification [5]. The blood plasma was placed in a chamber of the device, in which a flow was created with a shear rate of 40 s-1. Discrete microphotography of the aggregation process was performed at an interval of 20 seconds after the start of aggregation for 400 seconds. The degree and speed of aggregation were determined by the number of aggregates (rel.units) after 400 s and 160 s, respectively, after the start of the process using a special program [6]. The determination of platelet activation in the artificial shear flow was carried out similarly to the above-described method. However, K3EDTA-stabilized blood plasma was used for this purpose. The determination of platelet aggregation under these conditions makes it possible to judge the presence of receptors on their membranes in high-affinity (the presence of platelet activation) or low-affinity (the absence of platelet activation) states. In the presence of platelet aggregation, its degree was estimated by the

number of aggregates (rel.units) 400 s after the start of the aggregation process [7].

Statistical processing of the results was performed using variation statistics algorithms using Microsoft Excel 2007 and Statistica 6.1 for Windows.

RESULTS

As shown by the conducted studies, when the EDTA blood was stabilized, spontaneous aggregation of platelets in the control group under shear flow conditions was practically absent (Table 1). In children with IBD, the degree of platelet aggregation in these conditions exceeded the control values by 9 times, and continued to increase at discharge (Table 1).

Table 1. *Activation of platelets in children with IBD (at 400 sec)*

		Count of aggregates (rel. un.)
control (n=35)		1,89±0,91
IBD (n=120)	Before treatment	17,41±3,30 **
	After treatment	19,80±3,95 **

** p<0,05, ** p<0,001 — comparison to control*

During hospitalization of children with IBD, there was also a significant increase in the degree and rate of spontaneous (flow-induced) platelet aggregation (by 1.4 times and 1.3 times, respectively) (Table 2). Platelet aggregation indicators remained significantly elevated in IBD and as a result of treatment. In addition, a positive correlation was found between the number of aggregates formed during platelet activation and the same aggregation index during hospitalization of patients with IBD ($r = 0.50$, $p < 0.05$).

Table 2. *Spontaneous (flow-induced) aggregation of platelets in children with IBD*

		Count of aggregates (rel. un.)	
		160 sec	400 sec
control (n=35)		20,03±0,96	15,28±1,09
IBD (n=120)	Before treatment	25,60±0,92 **	21,05±1,08 *
	After treatment	23,60±1,11 *	19,73±1,25 *

** p<0,05, ** p<0,001 — comparison to control*

CONCLUSION

IBD in children is characterized by a significant increase in platelet activation and aggregation. The use of HBOT in the treatment of IBD contributes to a significant reduction in these processes.

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CHARACTERISTICS OF GUT MICROBIOTA IN AUTOIMMUNE DISEASES OF THE THYROID GLAND AND THE METHODS OF ITS CORRECTION

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ABSTRACT — Gut microbiota is considered as a pathogenetic factor of various diseases nowadays. The patients with autoimmune diseases are known to suffer from dysbiosis. There are studies in the modern literature that demonstrate changes in the composition of the gut microbiota in case of thyroid dysfunction. This review examines a contemporary view of the gut microbiota, its role in the development of autoimmune diseases. We investigated the interaction between the thyroid gland and the gut microbiota, its species composition in hypo- and hyperthyroidism. Possible methods of correction, including the use of pre- and probiotics and transplantation of fecal microbiota have been demonstrated.

KEYWORDS — gut microbiota, autoimmune diseases, thyroid gland, autoimmune thyroiditis, hypothyroidism.

INTRODUCTION

The prevalence of autoimmune diseases (AIDs) of the thyroid gland (TG), such as chronic autoimmune thyroiditis (AIT) and Graves' disease (GD) (diffuse toxic goiter), is estimated at about 5% [1]. AIT is a chronic inflammatory disease characterized by the production of specific autoantibodies against peroxidase (TPO-ab) and thyroglobulin (TG-ab) with the development of reduced thyroid function — hypothyroidism (HT) [1]. AIT makes about 46% of the total TG's pathology. According to the epidemiological data, the carrier state of TPO-ab is more common in the female population. There is also an increase in the incidence of TG's dysfunction with age. TG's hyperfunction is observed with GD and the main autoantigen is the thyroid-stimulating hormone (TSH) receptor [2]. At present, AIDs of TG are known to have a multifactorial etiology, including genetic, environmental factors, and food habits. Changes in the gut microbiota (GM) are one of the reasons for the development of AIDs. It has been proven that patients with AIT and GD have dysbiosis. Our review will consider the interaction between GM and the development of AIDs, the characteristics of GM composition in patients with TG's dysfunction and methods of its correction.

ROLE OF GM IN THE DEVELOPMENT OF AUTOIMMUNITY

The issue of the interaction between the composition of human GM and AIDs is actively studied nowadays. The intestine is the main site of interaction between pathogenic bacteria, food antigens and normal microflora. Epithelial cells, goblet cells producing mucous secretion, immune M-cells and lymphoid tissue are all the components of the intestinal barrier, the damage of which increases the host susceptibility to the effects of various agents [3]. The study of the intestinal wall structure showed similar changes in patients with type 1 diabetes and AIT, such as partial rarefaction of microvilli on the apical side of enterocytes, a decrease in their height [4]. A change in the GM profile can damage transmembrane proteins responsible for intestinal barrier integrity, such as ZO-1 and occludin [3]. The contact of intestinal immune cells with antigens and an increase in the intestinal permeability lead to inflammation and auto-aggression against the body's own cells.

Microbial imbalance is one of the pathogenetic factors in the development of AIDs. In dysbiosis, the ratio of regulatory Treg and inflammatory Th17 changes can also increase the susceptibility to pathogenic invasion [5]. Autoantibodies against the cell wall of Saccharomycecerevisiae have been found in patients with type 1 diabetes, rheumatoid arthritis (RA), systemic lupus erythematosus, antiphospholipid syndrome, and Crohn's disease [4]. Multiple sclerosis (MS) is an AID characterized by demyelination of nerve fibers. Analysis of the 16s ribosomal RNA gene showed the presence of dysbiosis in patients with MS. Colonization of Clostridium perfringens is associated

with a relapse of MS, and the produced toxins can cause microvascular complications leading to neuronal damage. Patients with severe dysbiosis and Sjogren's syndrome had higher disease activity and increased calprotectin level, which indicates the presence of an inflammatory process in the intestine. In the study of fecal microbiota in children with type 1 diabetes, representatives of the Blautia genus dominated, and their content positively correlated with the level of glycated hemoglobin and titers of antibodies to tyrosine phosphatase. The authors have suggested that it was the GM that influenced autoimmunity and may affect the development of type 1 diabetes [4]. C. aerofaciens may affect the pathogenesis of RA through various mechanisms, for example, by increasing the permeability of the intestinal wall, production of IL-17, and chemotaxis of neutrophils [6].

Thus, it can be assumed that changes in intestinal permeability and dysbiosis are the links in the pathogenesis of AIDs.

GM INFLUENCE ON THYROID FUNCTION. FEATURES OF GM COMPOSITION IN AIDS OF THE THYROID GLAND

In recent years, the study of the Thyroid-Gut-Axis has been of increasing interest to researchers. The formation of metabolically active triiodothyronine (T3) occurs with the participation of enzymes — deiodinases, localized in many body tissues, including the intestinal wall. The authors have suggested that in view of the large surface of the GIT, the contribution of T3 activated by the deiodinases to the total body pool is substantial. GM metabolic by-products, especially short-chain fatty acids (SCFA), together with TG's hormones, affect the differentiation of enterocytes and strengthen intercellular contacts in the intestinal wall [7]. GM participates in the metabolism of microelements, which are involved in the synthesis of hormones and the maintenance of normal TG function. With AIDs of the TG, there is a change in the levels of Lactobacillaceae and Bifidobacterium, the quantitative composition of which, in turn, positively correlates with the levels of selenium and zinc in the blood serum. It was suggested that disruption in the regulation of these microelements and GM composition could affect the course of AIT and GD [8].

There is evidence that microorganisms that are structurally homologous to human proteins through the mechanisms of molecular mimicry can cause AIDs of the TG. E.P. Kiseleva et al. have found Bifidobacteriumadolescentis, B. Longum, B. bifidum, and Lactobacillus plantarum on cells surfaces; these are the components that compete with human TPO-ab and TG-ab. The dependence between the presence of antibodies to B. bifidumand L. plantarum, and TPO-abwas also obtained [9]. Gram-positive bacteria, including strains of Lactobacillus rhamnosus, can activate the transcription factor NF-κB, which controls the expression of genes responsible for the immune response, either directly or through cytokines. In the study by Jiang W. et al. in patients with GD in GM analysis, an increase in the strains of Lactobacillus and Bacteroides was revealed, and in some patients, increased titers of TPO-ab were observed [10]. The authors have assumed that hormone imbalance in GD and changes in GM towards the predominance of Lactobacillus, which had similar amino acid sequences with TPO-ab and TG-ab led to the induction of proinflammatory signaling pathways with the development of autoimmunity.

GM can affect the predisposition to the development of HT in mouse models. As far back as in 1988, Penhale W.J. et al. demonstrated that injection of an antibiotic by sterile rats followed by injection of homogenized intestinal contents from rats raised under normal conditions increased their autoimmune susceptibility [10].

It is known that AIDs of the TG are characterized by dysbiosis. Some studies point out that there is a decrease in microbial diversity; others, on the contrary, note the small intestinal bacterial overgrowth syndrome (SIBO). The research of Yan HX. et al. showed that the species composition of GM was less diverse in patients with HT, and Phascolarctobacterium predominated [11]. In the group with GD, the diversity of microbial strains also decreased and there were changes in the GM profile in the form of an increase in the number of Bacilli, Prevotella, Lactobacillales, Megamonas, Veillonella and a decrease in Rikenellaceae, Ruminococcus, and Alistipescompared with healthy people [12]. In HT, there is a decrease in the motor function of the digestive system due to the accumulation of mucopolysaccharides and the development of the intestinal wall edema. Decreased GIT vermicular movement is one of the factors in the development of SIBO. The study involving 1809 people demonstrated that the most important factors in the development of SIBO were immunosuppression, conditions after gastric/intestinal resection and HT [13]. Lauritano E.C. et al. have found that about 54% of patients with HT suffered from SIBO [14]. Zhao F. et al. have found bacterial overgrowth and differences in the GM composition of patients suffering from HT and healthy individuals in the form of increased genera Blautia, Romboutsia, EubacteriumRoseburia, Ruminococcus, Fusicatenibacter, Dorea and Eubacterium and decreased levels of Fecalibacterium, Bacteroides,

Prevotella [15]. The change in GM correlated with clinical parameters, which could be actively used for the diagnosis of diseases. Another study also demonstrated a change in GM with a predominance of Shigella, Escherichia coli, and Parasutterella and a decrease in the content of Prevotella and Dialister [16].

It is known that GM affects the absorption of drugs. Impaired absorption of levothyroxine (LT4) can be caused by a change in the GM composition due to concomitant diseases of the GIT. There is evidence in the literature that the prevalence of coeliac disease-AID, which is characterized by gluten intolerance, with AIT, according to various sources, ranges from 5 to 24%. In the study by Bibbò S. et al. in patients with AIT and coeliac disease, a decrease in the level of Bifidobacterium was observed [17]. Virili C. et al. obtained that patients with isolated HT achieved normalization of the TSH level (on average, 1.02 mU/l) against the background of LT4 therapy at a daily dose of 1.31 μg/kg, while in patients with HT and coeliac disease during the same period of treatment with similar doses, higher TSH values (4.20 mU/l) were observed [18]. It was found that when correcting HT with concomitant coeliac disease, it was necessary to increase the daily dose of LT4, on average, to 1.96 μg/kg [19]. Patients infected with Helicobacter pylori also required large doses of LT4 to achieve compensation for HT.

CONCLUSION

GM plays an essential role in the human body, performing many functions: immunomodulatory, biosynthetic, protective, etc. With the advent of new non-culture-based research methods, it became possible to study the qualitative and quantitative composition of GM in various diseases. A change in the GM profile can be considered as one of the factors in the development of AIDs. The patients with autoimmune TG's dysfunction are known to suffer from dysbiosis. Evaluating the relevant literature data, it can be assumed that there is an interaction between hormone imbalance and GM. There is no doubt concerning the necessity for further clinical studies to identify the characteristics of the GM composition in AIDs of the TG. The detection of differences between normal and transformed GM can be considered as a diagnostic criterion for TG's diseases. Further study of GM correction methods will help to determine new strategies for the treatment and prevention of TG's diseases.

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BISMUT-INDUCED ONSET OF ACUTE TUBULOINTERSTICIAL NEPHRITIS: A CLINICAL CASE

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ABSTRACT — This article presents a clinical case of drug-induced acute TIN that developed in response to self-administered intake of bismuth tripotassium dicitrate. This clinical case was characterized by an acute onset, presence of urinary syndrome (low specific gravity of the urine and proteinuria), occurrence of azotemia in the setting of preserved diuresis, lack of hyperkaliemia, and quick and complete restoration of renal functioning after the discontinuation of a bismuth-containing drug. It can be suggested that the presented clinical case will allow for a better understanding of the causes and clinical features of acute TIN and the choice of the management tactics for patients with this pathology.

KEYWORDS — tubulointerstitial nephritis (TIN), medicinal nephritis, acute kidney damage, chronic kidney disease, bismuth trisalium citrate.

BACKGROUND

Drug-induced renal injury is one of the most acute problems in modern nephrology that is caused by the development of tubulointerstitial nephritis (TIN). As a rule, acute TIN is the main cause of *unspecified kidney injury* in patients with preserved diuresis and normal kidney sizes [1–5, 8]. TIN is an inflammatory condition of renal interstitium and tubules that is associated with the development of acute renal injury in 10–25% of cases. In 20–40% of cases, it leads to the development of chronic kidney disease (CKD). One of the drugs, which administration can be complicated by the development of TIN, is bismuth tripotassium dicitrate that exerts anti-ulcerous and anti-inflammatory effects combined with bactericidal activity against Helicobacter pylori. This drug is widely used in the treatment of ulcerous disease and chronic gastroduodenitis in the exacerbation phase [6–13]. This drug contains bismuth oxide that is primarily eliminated by kidneys. It can be suggested that the expansion of the knowledge on the peculiarities of this pathology can contribute to its timely diagnostics.

The study aimed to describe a clinical case of drug-induced acute TIN that developed in response to intake of bismuth tripotassium dicitrate and expand the doctors' knowledge about this pathology for the prevention, timely diagnostics, and treatment of acute TIN.

MATERIALS AND METHODS

The authors reviewed medical publications on clinical recommendations on drug-induced TIN for the past decade and analyzed the medical history of a patient from the Nephrology Department of the Tver Regional Clinical Hospital (Russia).

CASE DESCRIPTION

Patient K., 56 years old, female, was admitted to Nephrology department of the tertiary hospital complaining about pains in the epigastrium, feeling of abdominal borborygmi and bloating, loose stool up to 2–3 times per day, bilateral lumbar pain, and nausea.

Antibiotics were not prescribed in this case, because the patient self-medicated herself recognizing her symptoms as gastritis. She decided to consult a doctor only after taking the drug during 4 days, when her condition began to deteriorate.

The patient said that the condition developed quickly. The symptoms intensified within several days. The patient began to take bismuth tripotassium dicitrate 2 tablets twice a day without prescription. On day 4 after the beginning of the drug intake the patient developed face edema. The daily urine output decreased. During the examination at the local clinics, biochemical blood assay showed elevated levels of creatinine to 889.5 μmol/L and BUN to 21.6 μmol/L. The specified condition was defined as acute renal injury developed on day 4 after the drug intake. On day 6, the patient was hospitalized.

The patient's condition was satisfactory at admission to the hospital. Face edema and pastosity of the lower third ankles and feet were observed. The pulse was 80, rhythmic. BP was 16090 mmHg. The abdomen is soft, slightly painful in the epigastrium. The kidneys were not palpable. The diuresis was 1.0 L.

The clinical blood analysis revealed light normochromal anemia (hemoglobin 105 g/L), anisocytosis, light thrombocytopenia (167×10^9/L), eosinopenia,

ESR acceleration to 27 mm/h. Common urine analysis showed urine low specific gravity (1008), insignificant proteinuria (to 0.06 g/L), a large amount of flat epithelium, and 2–4 leukocytes in FOV. Biochemical blood assay at admission showed hyperazotemia (creatinine — 850 μm/L, BUN — 15 mmol/L). Ultrasonic investigation revealed diffuse alterations in the renal parenchyma with an increase in their size. ECG registered sinus bradycardia, horizontal electrical axis of heart, and signs of left ventricular overload. Esophagogastroduodenoscopy revealed esophagitis, mixed gastritis, duodenogastric reflux, and indirect signs of cholecystopancreatitis. EchoCG protocol showed insignificant hypertrophy of the basal area of the interventricular septum, left ventricular diastolic dysfunction, and preserved ejection fraction. Gastroenterologist and nephrologist examined the patient and evaluated the necessity of urgent hemodialysis. However, considering preserved diuresis, positive dynamics of creatinine levels decrease in the blood, and satisfactory condition, conservative management of the patient was chosen.

Clinical diagnosis was made based on the results of laboratory-instrumental tests. The primary diagnosis was drug-induced acute tubulointerstitial nephritis developed after intake of bismuth tripotassium dicitrate. Complications: moderate acute renal injury. Comorbid diagnosis: gastro-esophageal reflux disease: I degree esophagitis. Chronic gastroduodenitis in the exacerbation phase. Fatty hepatosis. Pancreatic lipomatosis. Hypertensive disease, II degree, high risk.

In the Nephrology Department, the patient received conservative therapy that included discontinuation of bismuth tripotassium dicitrate, indication of euphylline infusions (2.4% — 10.0 i/v drop infusion BID, daily), omeprazole (40 g i/v drop infusion before dexamethasone infusion), dexamethasone (16 mg i/v drop infusion daily with a gradual dose decrease to complete discontinuation), prednisolone (20 mg per os starting a week after the beginning of treatment at hospital), furosemide (40–60 mg i/v). During the therapy, the patient's condition improved and positive dynamics of laboratory parameters were observed.

At the patient's discharge a significant decrease in the levels of creatinine (to 150 μmol/L) and BUN (to 11 μmol/L) were observed. Clinical blood analysis showed normalization of hemoglobin, erythrocyte, and platelet levels, and a decrease of ERS to 16 mm/h. A common urine test revealed insignificant proteinuria (0.03 g/L). For outpatient therapy, it was recommended to take antiaggregants (pentoxifylline) 100 mg BID, per os, for 1 month. 1 month after the discharge, levels of creatinine and BUN were normalized.

DISCUSSION

The presented clinical case included the major manifestations of drug-induced acute TIN complicated by acute kidney injury developed in response to self-medication of bismuth tripotassium dicitrate. The clinical features of this case included acute onset (4 days after the self-medication), lack of oligo-anuria, presence of urinary syndrome (low specific gravity of the urine and proteinuria), increase in the level of creatinine in the blood with preserved diuresis, lack of hyperkaliemia, and quick and complete restoration of renal functioning after discontinuation of the bismuth-containing drug [10–13].

It can be suggested that the presented clinical case will allow better understanding of the causes and clinical features of acute TIN and the choice of management tactics for patients with this pathology.

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EXPRESS METHOD FOR TREATING NEGATIVE EMOTIONAL STATES IN DENTISTS

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ABSTRACT — This paper is aimed to examine the prevalence of such negative emotional states as tension, anxiety and depression among students and dentists (n=80). Herein, we developed the "Method for treating negative emotional states" and analyzed its effectiveness.
The data of questionnaire survey and determination of adaptive potential were assessed according to R.M. Baevsky. It was revealed that such parameter as decrease in the heart rate by 7 beats per minute could be used as a criterion to evaluate the effectiveness of treating negative emotional states in medical professionals. It has been proved that our original method is effective in managing negative emotional states, thus maintaining working ability, professional longevity and mental health among specialists and future doctors.

KEYWORDS — tension, anxiety, depression, pulse, adaptive potential, treatment of negative emotional states.

RELEVANCE

Medicine, in particular, dentistry is a type of human activity where negative emotions prevail, affecting individual's physical and psychological well-being [5]. Dentists in general are characterized by a low level of health and quality of life, high morbidity, one of the reasons for which is neuro-emotional overload and high levels of stress [3, 12].

Initially, the concept of *stress* originating in physiology characterized a non-specific reaction of the body in response to any adverse effect known as *general adaptation syndrome*. In reference studies of stress development among dentists, it is noted that several reasons could be distinguished in the activities of these specialists that allow them to be attributed to a risk group: constant competition, hard and painstaking work that is performed in a limited space of the oral cavity, etc.

These problems cause a high incidence of cardiovascular diseases, ulcers, colitis, neuromuscular pain, eye strain, and also affect family relationships, causing alcoholism, drug addiction, mental depression and even suicide [13]. Prolonged occupational stress, in turn, leads to the emergence of burnout syndrome [8]. Emotional burnout is considered as a breakdown in adaptation system developing as a result of prolonged work stress caused by exposure to various stressors, including physical, chemical, biological, social, and even one's own thoughts. It is also believed that stress and burnout are relatively independent phenomena. Thus, the key difference between burnout and stress is the causal factors. Stress occurs in a myriad of situations (e.g., war, natural disasters, illness, unemployment, various situations at work, etc.), while burnout manifests itself in connection with professional activities [6, 11]. Stress may not necessarily cause burnout, and conversely, stress can have a mobilizing effect on the human body.

Scientists have found that the practice activity of dentists at the age of 41–55 is characterized by predominance of negative emotions, maximum professional tension of personal adaptive resources that contribute to the formation of psychosomatic pathology in this contingent. At the same time, most of the dentists (64.3%) belong to the group of doctors who confirm the presence of psycho-emotional stress, 20.9% of which have signs of clear depletion of adaptation mechanisms [8].

When assessing the health condition in clinical practice, adaptive potential (AP) of circulatory system could serve as one of the effective health indicators, since cardiovascular system represent the primary adaptive mechanism of the whole organism that reacts in response to external conditions. It acts as a regulator of internal environment of the body, maintaining adequate blood supply and homeostasis of organs and tissues [7]. Considering the above mentioned evidence, we have chosen the well-known assessment of AP of circulatory system proposed by R.M. Baevsky [2].

Nowadays, there are many approaches for eliminating the breakdown of adaptation mechanisms such as psychotherapy, muscle relaxation, phyto- and drug therapy, etc. The majority of these therapeutic methods have one drawback: such treatment should be carried out for a long time, months and years, sometimes throughout life.

Therefore, it is of particular importance to develop short-term and adequate psychological methods aimed to prevent specific forms of psychological

tension and preserve professional longevity and mental health of specialists [1].

Aiming to solve the indicated problem, we have developed an express method to cope with such emotional states as tension, anxiety and depression and received a patent for this invention. The proposed method deals with embracing negative emotions via activation of the information processing system. It is effective, convenient, and most importantly, the therapeutic time does not exceed 3–5 minutes.

Aim of the research:

to evaluate the effectiveness of the author's method in treating emotional states in the study group.

The object of the research

includes negative emotional states and its manifestations in the practice of dentists and students of the dental faculty.

The subject of the research

is the breakdown of AP in the study group and its normalization by application of the author's method.

RESEARCH OBJECTIVES

1. To analyse the prevalence of emotional states in the study group.
2. Determine the AP of cardiovascular system in the study group according to R.M. Baevsky.
3. Eliminate the revealed emotional states using the author's "Method of treating emotional states".

RESEARCH MATERIAL

We examined 80 volunteers aged 21 to 64 years old, with mean age 34.16±0.97 years; 39 men and 41 women. 25% of examined volunteers were students of the Dental Faculty of Bashkir State Medical University (BSMU), 10% were orthodontists, 65% were students of the Department of Prosthetic Dentistry and Maxillofacial Surgery at the Institute of Additional Professional Education of BSMU.

RESEARCH METHODS

Testing, measurement of pulse, systolic and diastolic blood pressure was carried out twice in the examined volunteers: at rest while sitting in a chair (initial AP), and immediately after the intervention by the proposed author's method. It includes special actions with macrosaccades of eyes revealed during their smooth rotation and verbalized localization in the body of negative emotions [9]. For the objectivity of the experimental results, the volunteers received the treatment once.

Recognition of the functional state of volunteers was carried out on the basis of the test by V.V. Libinykh and A.V. Libinykh modified by A.F. Yermoshina called / *Constructive drawing of a person from geometric figures* [4] and the questionnaire proposed by us, as well as the analysis of AP results before and after treatment.

AP was determined by the formula:

$$AP = (0.0011^{*}HR) + (0.014^{*}SBP) + (0.008^{*}DBP) + (0.009^{*}BW) - (0.009^{*}H) + (0.014^{*}A) - 0.27,$$

where HR is the pulse rate per minute, SBP is the level of systolic blood pressure and DBP — diastolic blood pressure, BW — body weight, H — height and A — age [2].

For statistical processing of the obtained results, MS Excel 10.0 software was used. The calculation was carried out by nonparametric method χ^2 — Pearson's criterion with arithmetic mean values (M ± m).

RESEARCH RESULTS

Anthropometric characteristics among the subjects were as follows. The body height in females ranged from 152 to 178 cm, on average was 163.29±0.85 cm, while in males ranged from 165 to 202 cm, on average — 176.00±1.26 cm. The body weight in women varied from 44 to 85 kg, the average was 62.05 ± 2.06 kg, while in men the body weight was much higher ranging from 60 to 114 kg, the average was 80.23 ± 1.94 kg.

The mean SBP at rest and after treatment was 124.31±21.15 and 123.73±17.58 mm Hg, while DBP, respectively, was 77, 72±10.9 and 80.36±13.58 mm Hg. HR before and after exposure was significantly different ($\chi^2 = 0.000$): before — 79, 51±17.41 beats per minute, after — 72, 35±11.16. The difference was approximately 7 beats per minute [10]. Therefore, decrease in the heart rate could be used in further research as evidence of the effectiveness in treating negative emotional states.

Consequently, all examined volunteers were divided into 4 groups according to the presence of negative emotional states: I — those experiencing tension; II — anxiety; III — depression; IV — calmness.

According to the AP values, the functional state of the volunteers was determined and divided into the following groups:

I. AP below 2.6 corresponding to satisfactory adaptation of circulatory system;

II. AP between 2.6 and 3.09 corresponding to tension of adaptation mechanisms (practically healthy according to L.L. Miller [7]);

III. AP range from 3.10 to 3.49 corresponding to poor adaptation;

IV. AP of 3.5 and higher corresponds to adaptation failure.

Following the therapeutic intervention by the proposed author's method, the total number of volunteers experiencing negative emotional states decreased from 46.25% to 30.0% and was distributed as follows (Table 1).

Table 1. *Distribution of emotional states among the volunteers before and after intervention (n=80, χ^2=0,033)*

According to the intervention	Emotion				Total
	tension	anxiety	depression	calmness	
before	31,25%	15,0%	5,0%	48,75%	100%
after	21,25%	5,0%	3,75%	70,0%	100%

At the same time, there were no significant differences between men and women. The portion of respondents experiencing anxiety decreased most of all by approximately 66.7%, those experiencing tension — by 32.0%. The number of volunteers experiencing depression decreased by almost 25.0%, while those feeling calmness increased by 30.35%.

Depending on the results of AP before and after therapeutic intervention, all volunteers were distributed into the following groups (Table 2).

Table 2. *Distribution of AP before and after therapeutic intervention into the following groups (n=80, χ^2=0,000)*

Group of AP	Before therapeutic intervention	After therapeutic intervention			
		I	II	III	IV
I	50,0%	87,5%	12,5%	–	–
II	23,75%	26,32%	73,68%	–	–
III	22,5%	5,5%	22,22%	72,22%	–
IV	3,75%	–	–	33,33%	66,66%

Before the therapeutic intervention, half of the volunteers were included in the group I according to the AP values, where the adaptation of circulatory system was satisfactory. The rest of the respondents were distributed as follows: 23.75% of the respondents entered the II and 22.5% — III group, corresponding to tension and unsatisfactory adaptation. The group IV was the smallest one and included those experiencing a breakdown in adaptation.

After the therapeutic intervention, the following results were obtained: a large proportion of the studied group I (87.5%) remained in the same group characterized by satisfactory adaptation with sufficiently high functional capabilities of the organism. The remaining 12.5% of the respondents from the first group experienced stress and moved to the second group. 26.32% of the respondents from the group II improved their indicators and moved to the group I with satisfactory adaptation. Meanwhile, the remaining 73.68% of cases from the second group remained the same. 22.22% of respondents from group III moved to group II, 7.69% — to group I, thereby improving their AP indicators. 33.33% of respondents from group IV with "failure of adaptation" moved to the III group with unsatisfactory adaptation, and in terms of the numerical AP indicators improving their condition.

To conclude, a single treatment of negative emotional states with the proposed author's method resulted in the improvement of adaptive capabilities in 13.75% of respondents. At the same time, there were no cases of deteriorated AP parameters after the therapeutic intervention as compared to the baseline levels.

CONCLUSION

1. Negative emotional states such as tension, anxiety, depression are widespread among dentists.
2. A statistically significantly specific effect of our method on the body functional status has been confirmed.
3. A decrease in the heart rate in the adult population by 7 beats per minute can be used in scientific research as a reliable criterion for the effectiveness of treatment of emotional states.
4. The proposed therapeutic intervention method is effective in treatment of such negative emotional states as tension, anxiety and depression. It enables to

maintain professional longevity and mental health of dental specialists and future doctors.

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IMMUNOLOGICAL ASSESSMENT OF THE STRESS RESPONSE IN PATIENTS WITH INFLAMMATORY POSTPROTHETIC COMPLICATIONS

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ABSTRACT — Post-prosthetic complications during dental implantation are accompanied by pain symptoms leading to disorders of the psychoemotional state. All this influences the behavior of patients. In addition, psychoemotional stress is often a factor of provocation and persistence of the complications. The presence of a stress state in the identified pathology, as well as the influence of various therapies on the treatment of post-prosthetic complications, is reflected in the dynamics of changes in the concentrations of both catecholamines (epinephrine, norepinephrine) and glucocorticoids (cortisol) — hormones of the medulla and the adrenal cortex. THE AIM: to conduct an immunological analysis of the stress response in patients with post-prosthetic complications during dental implantation. MATERIALS AND METHODS: The study was performed in 120 patients with post-prosthetic complications during dental implantation before and during treatment: Group I (control) — 30 patients treated with the conventional therapy; group II — 30 patients treated with ozone therapy in addition to the conventional therapy; group III — 30 patients treated with transcranial electrical stimulation in addition to the conventional therapy; Group IV — 30 patients treated with a combination of conventional therapy, ozone therapy and transcranial electrical stimulation. The concentration of epinephrine, norepinephrine, cortisol, alkaline phosphatase, and the Garkavi index were evaluated. RESULTS: The change in these indicators after the treatment indicates the normalization of the level of the studied enzymes — markers of bone homeostasis, which is confirmed by an improvement in the clinical picture in the oral cavity. CONCLUSION: changes in immunological parameters objectively reflect the psychoemotional state of patients. The nature of changes in the hormonal stress response to the treatment of post-prosthetic complications indicates the effectiveness of the therapeutic regimens used, and, as a result, a decrease in both pain symptoms and psychoemotional stress.

KEYWORDS — epinephrine, norepinephrine, inflammation, implants, treatment.

RELEVANCE

As a response to the impact of such aggravating factors as pain, blood loss [7], mechanical tissue damage [1–5], hypoxia [6], increased free radical oxidation [8, 9], a non-specific neuroendocrine reaction appears [10]. Consequently, the influence of different types of therapeutic approaches for the treatment of post-prosthetic complications is reflected in the dynamics of changes in serum concentrations of both catecholamines (epinephrine, norepinephrine) [11] and glucocorticoids (cortisol) — hormones of the medulla and the adrenal cortex [12]. Determining the level of cortisol is an important indicator in the diagnosis of a stressful situation. Increased cortisol levels are observed with a decrease in psychomotor activity, sleep disorders, and depression. The reference values of cortisol in the blood serum are 200–700 nmol/l at 8.00 a.m. and 55–250 nmol/l at 20.00 pm. The difference between the concentrations in the morning and in the evening should be at least 100 nmol/l.

The biochemical catalyst for stress is epinephrine, which is released by the adrenal glands. It activates glycogenolysis in muscle cells, rises heart rate and blood pressure, dilates blood vessels of heart muscles and stimulates the production of ACTH and glucocorticoids. The level of adrenaline in the blood depends on the tone of the sympathetic system. Its content in the blood plasma should not exceed 112–658 pg/ml.

The mediator of the sympathetic nervous system is the hormone of the adrenal medulla — norepinephrine. A decrease in the level of the ratio of norepinephrine to adrenaline in the urine of patients may indicate their depressive states. In severe depression, the level of the norepinephrine metabolic product - 3-methoxy-4hydroxyphenylglycol is often reduced, and the amount of the serotonin derivative — 5-hydroxyindolacetic acid is reduced in the cerebrospinal fluid of these patients. An increase in the daily excretion of epinephrine and a decrease in the release of norepinephrine indicate a depressive phase of manic-depressive psychosis, and a manic state is characterized by a multiple increase in the excretion of norepinephrine. Both in mania and in depression, the blood content of adrenaline in patients is increased.

A decrease in the level of norepinephrine leads to an increase in the level of cortisol, and a deficiency of serotonin, which is accompanied by a disruption in regulation of cortisol secretion. The lack of both leads to disruption of circadian rhythms in the secretion of cortisol. The reference values of the content of norepinephrine in the blood plasma should not exceed 10 pg/ml.

The aim of our study was to evaluate the stress response of patients with inflammatory postprothetic complications against the background of various pharmacotherapy methods according to immunological parameters.

MATERIALS AND METHODS

The study included the diagnosis and follow-up of 120 patients aged 18–44 years (WHO average age) with post-prosthetic complications during dental implantation before and during treatment. The patients were randomized into groups according to the treatment regimen: Group I (control) — 30 patients who were treated with conventional treatment; group II — 30 patients who were treated with ozone therapy in addition to conventional treatment.; Group III — 30 patients, in the complex treatment of which, in addition to traditional therapy, transcranial electrostimulation was performed; group IV — 30 patients, whose complex treatment included a combination of traditional therapy, ozone therapy and transcranial electrostimulation.

Based on the general and biochemical blood analysis, the indicators that allow us to assess the stressful situation in the study groups (cortisol concentration, alkaline phosphatase, Garkavi index, epinephrine, norepinephrine) were determined.

General and biochemical blood tests were performed using a laboratory test. The leukocyte formula, the concentration of glucose, creatinine, total protein and other indicators were studied. The type of adaptive response (stress, quiet activation, re-activation) was determined by the percentage of lymphocytes in the peripheral blood, taking into account age. The Garkavi index was used as a basis.

Garkavi index = lymphocytes (%) / segmental neutrophils (%);

In healthy adults, this indicator is within the values of 0.3–0.5.

To determine the alkaline phosphatase (ALP) in the blood serum, a spectrophotometric method was used using reagents from the company "Human" (Germany) and a calibration curve constructed from a standard solution of p-nitrophenol. The method is based on the following reaction:

alkaline phosphatase

p-nitrophenyl phosphate + H_2O – " p-nitrophenol+ phosphate

The measurement was carried out at a temperature of 37° C and a wavelength of Hg 405 nm (400–420 nm). The average change in optical density per minute (AA/min) was calculated. The activity of the alkaline phosphatase was calculated by the formula

E / l = AA/min x 2757

Blood samples of the patients in the volume of 5 ml were obtained from 6.00 to 10.00 a.m. on an empty stomach (Fig. 1).

On the eve of the examination, patients were advised to refrain from overeating, eating salty, spicy, fatty foods, exclude alcohol, and do not smoke an hour before the examination. The biomaterial was collected before undergoing instrumental examination and physiotherapy. Venous blood obtained without anticoagulants in a centrifuge glass tube is settled in it at room temperature, then centrifuged for 10 minutes at 2500 rpm.

Repeated blood tests to monitor the dynamics of indicators were carried out under the same conditions (time, food regime) and in the same laboratory, since the blood sampling algorithm, study methodology and reference values (norms) may differ significantly in different medical institutions.

The results were evaluated on day 3, 7, 14, and 1 month later. Two-factor analysis of variance (RANOVA) with the Newman-Keuls post-hawk test was performed; * — $p<0.05$; ** — $p<0.01$;*** — $p<0.001$.

The clinical trial was approved by the Regional Ethics Committee, Protocol No. 2115/1-2019 of April 19, 2019.

RESULTS

Before the start of therapy, no statistically significant differences in the studied parameters were found between the groups. At the same time, for all certain hormones, a statistically significant decrease was observed as a result of therapy. The study revealed a relative increase in the concentrations of hormones (epinephrine, norepinephrine, cortisol) before the start of therapy, which reflects the overall clinical picture of the stress response. At the same time, there were no statistically significant differences between the groups before the start of therapy. The content of epinephrine varied in the range of 127.047±19.4024 pg/ml, norepinephrine 381.3607±40.2137 pg/ml, cortisol 430.62±40.275 nmol/L.

When determining the content of epinephrine in all groups, there was a decrease in its concentration at a more intense rate following the combined method of treatment (Fig. 2).

The decrease in the concentration of epinephrine in the blood serum occurred up to the 5th control point (1 month). At the same time, the treatment regimen combined with ozone therapy showed a significantly greater decrease (59.348 vs 73.438; p<0.01), but for groups III and IV, the differences were even higher (59.348 vs 58.348 and 55.406, respectively, p<0.001). At the same time, in the nearest follow-up period (up to 14 days), the lowest values were typical for groups II and IV (p<0.01 and p<0.001, respectively). For norepinephrine, similar dynamics were observed, while the differences with the comparison group (conventional therapy) were less pronounced. At the same time, the dynamics of the greatest decrease in the concentration of norepinephrine in the immediate follow-up period (3–30 days) was shown for the treatment regimen combined with ozone therapy (3, 14, 30 days — p<0.01; 7 days — p<0.001), similar values were found for group IV (3, 7, 14, 30 days — p<0.01). The dynamics of changes in concentrations characteristic of epinephrine and norepinephrine and the revealed tendency to decrease in the near-term follow-up were also shown for changes in serum cortisol concentrations, regardless of therapeutic regimens. And by day 14 of the therapy they were significantly lower relative to the start of therapy and 3 days (p<0.05). This was followed by a slight increase in these parameters. The inter-group differences for cortisol were similar to those for norepinephrine, but the lowest values were observed at the 4th control point for all groups. The dynamics of the greatest decrease in cortisol concentration in the nearest follow-up period (3–30 days) was observed in group IV (7, 14, 30 days — p<0.01; 3 days — p<0.001). Similar values were found for group II (3, 7, 14, 30 days — p<0.01). An increase in the Garkavi index which reflects the relationship between the humoral and cellular components of immunity, an assessment of the stress state, as well as an assessment of the adaptive responses of training and activation, was found in all study groups, whereas no significant differences were found between the groups. The average value was 0.687±0.05. In the course of all types of therapy normalization of the index was achieved by 14 days. A stable decrease in the index in all groups during the first 2 weeks indicates a decrease in the active inflammatory process. Thus, the nature of changes in the hormonal stress response to the treatment of post-prosthetic complications indicates the effectiveness of the therapeutic regimens used, and, as a result, a decrease in both pain symptoms and psychoemotional stress. In the near term of observation, there is a significant decrease in the concentrations of the studied hormones (catecholamines and corticosteroids), normalization of the values of the Garkavi index (Table 1–4).

Fig. 1. *Venous blood sampling*

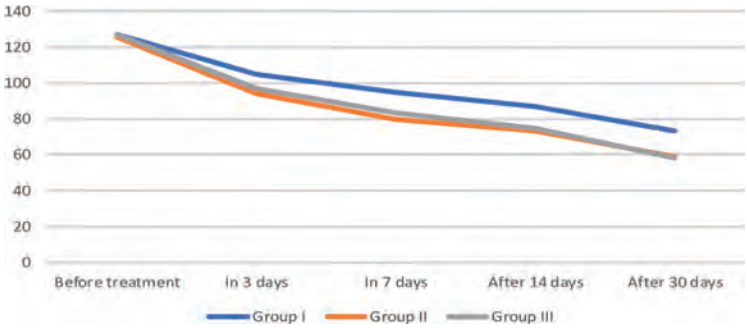

Fig. 2. *Dynamics of changes in the concentration of epinephrine during 1 month of observation*

The concentration of epinephrine in group I significantly decreased until 1 month after the start of therapy.

Evaluating the Garkavi index, a stable decrease in the index was revealed in all groups for 2 weeks which indicates a decrease in the active inflammatory process. Normal values of the index were maintained on day 30.

The parameters of epinephrine concentration in group II (in the complex treatment, ozone therapy was additionally used), group III (additional use of transcranial electrical stimulation) and in group IV (a combination of methods of groups 1–3) behaved similarly to the first, but the decrease to 1 month from the start of therapy was more pronounced.

Thus, the analysis of the dynamics of the content of stress hormones in the blood serum showed a characteristic and significant decrease in the concentrations of hormones up to 1 month, followed by a slight increase in concentrations. These trends indicate the effectiveness of the therapeutic regimens used, and as a result, a decrease in both pain symptoms and psychoemotional stress. And taking into account the relatively

Table 1. *Immunological examination of blood in patients of group I*

Signs/ deadlines	Cortisol		SCHF		Index Garkovi		Adrenaline rush		Norepinephrine	
	M	SD	M	SD	M	SD	M	SD	M	SD
Before treatment	420,400000	38,760000	118,00000	10,75200	0,687400	0,05000	127,35840	20,14200	381,348600	40,354800
3 days	362,286300	30,698040	122,59390	8,75520	0,561200	0,03470	105,34580	16,35400	362,286300	20,252820
7 days	266,626500	26,680250	122,56790	6,32016	0,523040	0,03280	94,86240	16,43800	266,626500	22,801480
14 days	215,480000	19,364800	133,25590	9,21600	0,437300	0,03760	86,96423	15,34600	215,480000	18,258790
1 month	29,881800	20,482120	145,99580	9,02880	0,450300	0,05380	73,43840	13,24580	219,881800	17,504430

Table 2. *Immunological examination of blood in patients of group II*

Signs/ deadlines	Cortisol		SCHF		Ind.Garkovi		Adrenaline rush		Norepinephrine	
	M	SD	M	SD	M	SD	M	SD	M	SD
Before treatment	435,240000	42,180000	117,000	8,96000	0,686900	0,057400	126,78210	19,823760	382,103500	39,246800
3 days	340,027200	30,742150	126,677	11,85030	0,527028	0,0346653	93,81634	15,079810	343,927200	19,546140
7 days	245,058900	26,718590	127,644	9,59310	0,4804876	0,0327572	80,35800	15,348600	244,058900	22,005870
14 days	194,078500	15,395500	141,248	9,21600	0,399419	0,035524	73,34680	15,103530	196,078500	17,621690
1 month	198,124800	20,511550	148,918	10,72170	0,4114996	0,0537462	59,34861	13,036520	200,124800	16,893650

Table 3. *Immunological examination of blood in patients of group III*

Signs/ deadlines	Cortisol		SCHF		Ind.Garkovi		Adrenaline rush		Norepinephrine	
	M	SD	M	SD	M	SD	M	SD	M	SD
Before treatment	429,090000	40,740000	120,000	10,2400	0,689800	0,054600	127,146300	19,632050	380,904600	40,348030
3 days	343,209000	26,046490	127,594	11,2860	0,526056	0,03283619	97,346800	16,914630	345,209000	16,588630
7 days	247,097700	22,244850	128,568	12,0960	0,5045652	0,04050112	83,346000	15,346800	249,097700	18,676170
14 days	196,486000	14,215370	142,256	9,8503	0,4018549	0,0450433	75,348600	14,957470	198,486000	14,955360
1 month	199,642800	18,380180	149,996	9,2160	0,4154934	0,05091029	58,348120	12,910440	202,642800	14,337480

Table 4. *Immunological examination of blood in patients of group IV*

Signs/ deadlines	Cortisol		SCHF		Ind.Garkovi		Adrenaline rush		Norepinephrine	
	M	SD	M	SD	M	SD	M	SD	M	SD
Before treatment	437,750000	39,420000	119,000000	12,80000	0,687045	0,052400	126,907500	18,001710	381,086200	40,905370
3 days	336,507200	30,423910	131,421800	12,09600	0,5170252	0,02936557	94,354860	15,509960	342,119200	18,921110
7 days	242,522000	26,442000	132,425000	9,21600	0,4854849	0,03622036	76,365860	15,124030	245,351900	21,302180
14 days	192,069400	15,236130	144,463700	9,59310	0,3994168	0,04028245	69,648560	13,715330	195,750400	17,058190
1 month	196,073800	20,299220	154,495900	9,59310	0,4111898	0,04552932	55,406310	11,838300	199,851300	16,353440

maximum decrease in the concentrations of the presented hormones for group IV, combining traditional therapy with ozone therapy and transcranial electrical stimulation, for which there were large inter-group differences relative to traditional therapy both in the near and long-term follow-up periods, it can be argued

that this scheme was most effective in the treatment of post-prosthetic complications.

An increase in the content of alkaline phosphatase up to 1 month and a further retention of the values in the plateau phase may indicate that the use of therapy, especially combined with ozone therapy and transcranial electrical stimulation, activates the processes of connective tissue synthesis.

The results of the obtained values indicate a high level of psychological stress in groups of patients with different therapy for post-prosthetic complications of dental implantation at different time intervals. Based on the data obtained, it can be argued that the changes in the concentrations of epinephrine, norepinephrine, alkaline phosphatase, and cortisol detected at the beginning of therapy fully fit into the picture of the immune response under psychophysiological stress and objectively reflect the changes in the psychophysiological state of patients with postprothetic complications.

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DEVELOPMENT OF A BIODEGRADABLE MATERIAL FOR BONE REGENERATION BASED ON SILICON COMPOUNDS

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ABSTRACT — In the course of this study we established that the laboratory sample in granulated form developed by us has a stimulating effect on the growth and proliferation of osteoblasts. Analysis of the data (CT, MRI) of the biological material obtained from the animals withdrawn from the experiment showed a statistically significant replacement of the artificial defect with bone tissue with signs of restoration of the cortical plate. The analysis of the CT results indicates that this method is sufficiently informative in assessing the regeneration of the trabecular structure of bone tissue. The study (MRI) of the bone material obtained from the animals participating in the experiment showed that when the osteoinductive material was integrated, the defect zone was replaced by 90%. In the projection of the defect, the newly formed tissue of a homogeneous structure was determined, corresponding in density to bone tissue with a high degree of mineralization. There was a complete restoration of bone tissue in the projection of the defect.

KEYWORDS — osteoplastic materials, bone defects, bone powder, dental implantation.

INTRODUCTION

One of the most urgent problems of traumatology, orthopedics, surgical dentistry and maxillofacial surgery is the problem of replacing bone defects. A distinctive feature of these problems is the lack of expression of the natural regenerative processes of bone tissue. Given that the spontaneous restoration of the integrity of bone tissue in the area of the defect is either impossible or excessively long, therapeutic measures are based on the use of various bone-substituting (osteoplastic) materials, which are designed to enhance the activity and effectiveness of the recovery process.

The most effective of the osteoplastic materials is the patient's own (autogenous) bone tissue. However, the need to expand or create a new surgical field to obtain fragments of the patient's own bone tissue from donor sites (ilium, ribs, mandible), complication and lengthening of surgical intervention, increased risk of complications, the specifics of the somatic status and preferences of patients impose restrictions on the use of autogenous bone tissue and force the use of products available on the market.

However, the vast majority of osteoplastic materials on the market are a kind of *biologically active additives* — they only supply structural components necessary for the formation of bone tissue in the defect zone, therefore they have low or extremely moderate effectiveness.

Purpose of the study

The purpose of this study was to establish the regularities of morphological changes in bone tissue of laboratory animals for correction of defects in the maxillofacial region using osteoplastic material.

MATERIALS AND METHODS

Examination (CT, MRI) of biological material obtained from animals were used in the experiment. The results of the experiment were registered.

In our experiments we fully adhered to Ethical Guidelines for the Use of Animals in Research (EU Directive 2010/63/EU).

The object of the CT study was the bone tissue of the lower jaw of a rabbit obtained 90 days after the integration of experimental osteoplastic material, fixed in 10% buffered formalin in accordance with the rules for pathomorphological and histological studies.

There was a complete restoration of bone tissue in the projection of the defect (Fig. 1). The newly formed bone looked immature: the bone beams were uneven in shape and size, randomly arranged.

In CT with the reconstruction of the three-dimensional image, the replacement of the defect with bone tissue with the restoration of the cortical plate was determined. The CT data allowed us to visualize the formation of the trabecular structure of bone tissue in the projection of the defect at a given regeneration period of 90 days (Fig. 2).

The analysis of CT results in bone regeneration showed its informative value in assessing the trabecular

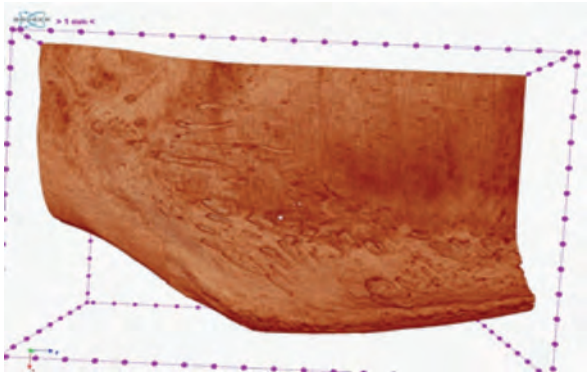

Fig. 1. Three-dimensional 3D model of bone tissue in color

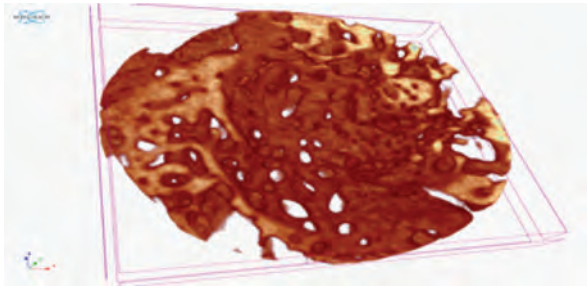

Fig. 2. Formation of the trabecular structure of bone tissue. Three-dimensional 3D model in color

structure of bone tissue. The reconstruction of three-dimensional images made it possible to determine the spatial localization of structures in the projection of the bone defect due to the absence of the overlap effect, to outline the surface structures in relief and to evaluate the complex three-dimensional relationships between bone and soft tissue fragments.

The study (MRI) of the biological material obtained from the animals in the experiment showed that when the osteoinductive material was integrated, the defect zone was replaced by 90%.

In the projection of the defect, the newly formed tissue of a homogeneous structure was determined, corresponding in density to bone tissue with a high degree of mineralization. There was a complete restoration of bone tissue in the projection of the defect (Fig. 3). The newly formed bone looked immature: the bone beams were uneven in shape and size, randomly arranged.

Histological examination of the bone in the defect area of the lower jaw of rabbits in the time interval from 14 days till 3 months has shown that the healing of the bone wound, the features and the rate of formation and maturation of bone regenerate were associated with the osteoinductive and osteoconductive properties of the experimental drug.

Fig. 3. MRI, three-dimensional image reconstruction

CONCLUSION

Control studies of the biological material obtained from the animals used in the experiment confirmed our research.

The results of computed tomography revealed the persistence of the tendency to increase the density of regenerate in animals. By the end of the experiment, the bone defect was completely closed. Along the edges of the former opening, the trabeculae were partially resorbed and the bone began to transform into a lamellar bone with a developed system of haversoid channels. Bone tissue rearrangement in the area of the formed regenerate in animals with local administration of the experimental drug occurred at an earlier time, which was determined by the density of the regenerate, this is a sign of a sufficiently high degree of biocompatibility of bone tissue and experimental osteoplastic material (Fig. 4).

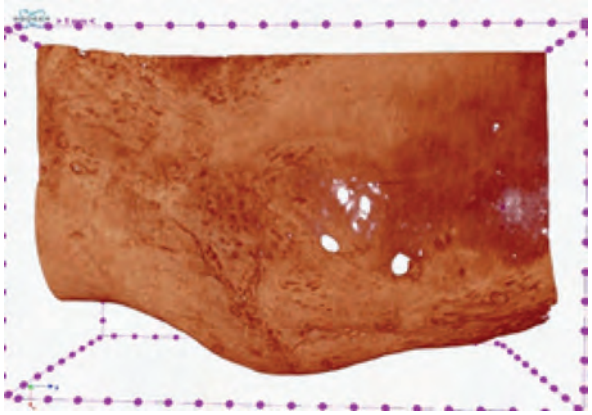

Fig. 4. 3D volumetric model in color

The MRI results confirmed that the implanted material corresponds to the bone tissue in density and structure, and the regenerate density is uniform (Fig. 5).

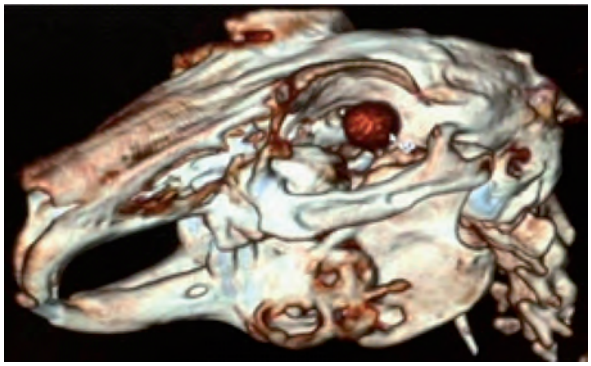

Fig. 5. MRI of the bone tissue of the lower jaw

On the 14th day after the operation, the formation of immature bone tissue began, by the 20th day, the bone tissue already filled most of the volume of the defect (two-thirds). At the same time, there was a certain *maturation* of the regenerate, characterized by an orderly arrangement of fibrous structures, although it still consisted of osteoid beams. Part of the regenerate had a *chondroid* structure. 1 month after the operation, the defect was almost completely filled with bone regenerate, the bone marrow was absent (Fig. 6). After 2 months, the reticulofibrous bone tissue matured and was partially replaced by lamellar bone. After 3 months, the bone tissue of the regenerate has already fully matured, compacted, clear lines of adhesion appeared, and osteons were formed (Fig. 7).

The bone regenerate was similar in structure to the spongy bone of the operation area, only slightly different in thickness and pronounced signs of bone remodeling.

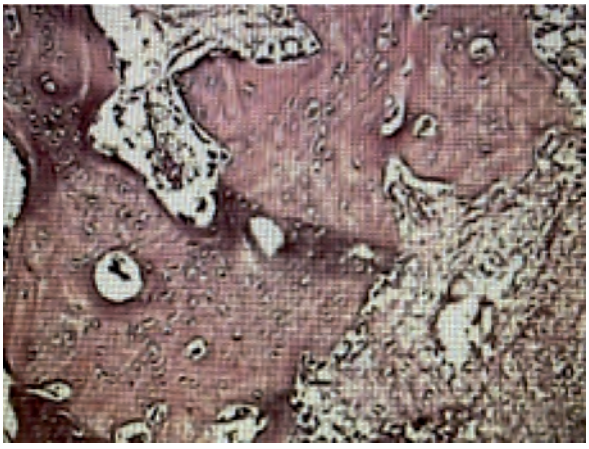

Fig. 6. The defect is filled with maturing regenerate, the bone compaction looks weak. Stained with hematoxylin and eosin

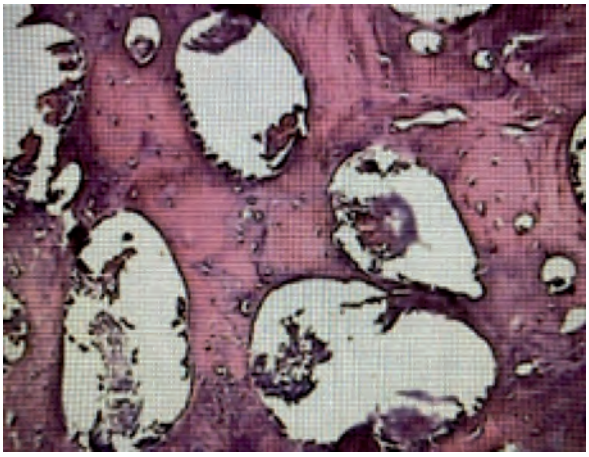

Fig. 7. Relatively mature bone regenerate with compaction phenomena. Stained with hematoxylin and eosin

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THE PREVALENCE OF NON-CARIOUS TOOTH DEFECTS ASSOCIATED WITH INDUSTRIAL EXPOSURE IN RESIDENTS OF PENZA REGION (RUSSIA)

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ABSTRACT — Aim of the study: to assess the level of dental pathology in Penza Region (Russia) and estimate the impact of the industrial environment. Materials and methods: A clinical examination and analysis of the frequency of enamel erosion, fluorosis and hypoplasia, wedge-shaped defect and multiple enamel cracks were carried out among the main professional groups of the region's population — workers of the machine-building and timber processing industries in the Penza region. Results and Discussion. Among the residents of Penza region, the frequency of enamel erosion was 4.0%, pathological tooth wear — 7.1%, wedge-shaped defect — 6.1%, multiple enamel fractures — 11.1%. There is a high frequency of non-carious tooth defects that occur before teething. In patients working in the machine-building industry, a high frequency of pathological tooth wear was noted (24,5%); in workers of the timber processing industry, it was 2 times lower (12,6%), as well as a high frequency of wedge- shaped defects, enamel erosion and multiple enamel cracks — all this was 2–2.5 times higher compared to similar indicators in patients from agricultural populations. However, the prevalence of dental fluorosis and hypoplasia was almost the same as in the agricultural population. Conclusion: Penza region has a high prevalence of dental fluorosis and hypoplasia. At the same time, the influence of industrial environment on the incidence of various types of non-carious dental pathology in workers of machine-building and timber processing industries in Penza region was established.

KEYWORDS — non-carious tooth defects (NCTD); prevalence; intensity; concentration of fluoride.

INTRODUCTION

In addition to caries, a significant place among dental diseases is occupied by non-carious enamel lesions: various types of hypoplasia (systemic, local, focal), imperfect amelogenesis, dentinogenesis, Stanton-Candepon syndrome, as well as fluorosis.

Non-carious lesions of the enamel of the teeth, which have a congenital character, are formed in the antenatal period. The physiological course of pregnancy is of great importance in the biosynthesis of normal enamel and its preservation [6]. The differential diagnosis of enamel abnormalities should be carried out with various forms of dental fluorosis, which also manifests itself as a variety of white, yellowish or brown spots on several or all teeth [5]. Fluorosis is an endemic disease that occurs as a result of an increased content of fluoride in drinking water. The exact mechanism of the occurrence of fluorosis is not yet fully understood. It is believed that fluorosis increases the content of fluoride in saliva and on the surface of enamel. It was shown that the prevalence of fluorosis directly depends on the content of fluoride in drinking water: at a fluoride concentration of 0.8–1.0 mg/l, the prevalence of fluorosis is 10–12%, 1.0–1.5 mg/l — 20-40%, 1.5–2.5 mg/l — 30–40%, over 2.5 mg/l — more than 50% [2]. Thus, the intensity of fluorosis in the population in endemic foci can be different: from mild lesions to significant changes in the teeth. This means that at the same concentration of fluoride in water, the body can react differently to its intake [1].

METHODS

A clinical examination and analysis of the frequency of enamel erosion, fluorosis and hypoplasia, wedge-shaped defect and multiple enamel cracks were carried out among the main professional groups of the region's population-employees of the machine-building and timber processing industries of the Penza region.

In this study, we analyzed dental examination data from 200 subjects aged 25 to 75 years (56 men and 144 women), of which 16 people were aged 21 to 30 years, 40 people — 31 to 40 years, 24 people — 41 to 50 years, 80 people — 51 to 60 years, 24 people — 61 to 70 years, 16 people — 71 to 80 years.

RESULTS

Among the native residents of Penza region (represented by the group of the rural population almost completely, without the share of the alien population), the frequency of enamel erosion was 4.0%, pathological tooth wear was 7.1%, and the wedge-shaped defect was 6.1%. Occurrence of multiple fractures of the enamel was even more common — 11.1%. Attention is drawn to the high frequency of non-carious dental diseases that occur before eruption (the incidence of

dental hypoplasia was 14.1%, dental fluorosis 24.2%) — the region is endemic for fluorosis.

Interesting differences in the incidence of major non-carious tooth defects are provided by an analysis of respondents from the major occupational groups of the population – workers of machine-building and woodworking industries. In patients from the machine-building industry, there was a high incidence of pathological tooth wear (24.5%, and in workers of the timber processing industry — 12.6%, 2 times lower), as well as a high incidence of wedge–shaped defects (13.3%), enamel erosion (11.4%) and multiple enamel cracks (20.9%) — all this was 2–2.5 times higher compared to similar indicators in patients from the agricultural population.

However, the prevalence of fluorosis and dental hypoplasia was almost the same as in the agricultural population — 13.3% and 11.4% (even the incidence of fluorosis was slightly lower, which indicates a greater proportion of the non-indigenous population of the region in the group of workers in the engineering industry). In patients from workers of the timber processing industry, a high frequency of many non-carious diseases of the teeth was also noted, but less than in workers of the machine-building industry: in particular, the frequency of occurrence of pathological tooth wear was 12.6%, wedge-shaped defect — 8.0%, enamel erosion — 8.0%, multiple enamel cracks — 14.9%. High prevalence rates of fluorosis (13.8%) and dental hypoplasia (8.0%) were observed.

Overall, it was noted that on the first place in frequency of non-carious tooth defects among the workers of machine-building industry was pathological tooth wear (24,5%) followed by multiple fractures of the enamel (20,9%) and wedge-shaped defect and fluorosis of the teeth (13.3%), and more rarely — the erosion of enamel hypoplasia of the teeth (11.4%). Among the workers of the timber processing industry, multiple enamel cracks (14.9%) and dental fluorosis (13.8%) were most often encountered, less often — pathological tooth wear (12.6%), wedge-shaped defect, enamel erosion, tooth hypoplasia (8.0% each). In respondents employed in agriculture, non-carious pathology was mostly manifested as fluorosis (in 24.2% of respondents in this group) and hypoplasia of teeth (14.1%). Multiple enamel cracks (11.1%), pathological tooth abrasion (7.1%), wedge-shaped defect (6.1%) and enamel erosion (4.0%) were less common.

DISCUSSION

In other words, work in the machine-building industry of the Penza region features the highest incidence frequency of certain types of non-carious tooth defects. In this population pathological tooth wear was 2 times higher than in workers of the timber processing industry and almost 4 times higher than in people employed in agriculture. The wedge-shaped defects and enamel erosion was also 1.5 times higher compared with workers of the timber processing industry and 2 times higher than in respondents employed in agriculture. On the other hand, agricultural populations had a higher incidence of dental fluorosis and hypoplasia, which is associated with a higher proportion of the native population and a smaller proportion of the alien population. The leading role in the frequency of different types of non-carious pathology among workers of the machine-building and wood-processing industries is occupied by pathological tooth wear, wedge-shaped defect and multiple enamel cracks, which proves the influence of industrial hazards.

Analysis of correlation between the frequency of non-carious tooth defects and the seniority of different groups of workers allowed us to determine the dependence of this disease from exposure to occupational factors. Thus, high indicators of strong correlation relationship were noted in the groups of workers from machine-building and timber processing industries in relation to the pathological tooth wear (0.76 and 0.70 points) and the wedge-shaped defect (0.68 and 0.64 points); these indicators were almost 2 times lower for those employed in agriculture (0.37 and 0.30 points). In addition, high values of the correlation with seniority were demonstrated in respondents employed in the machine-building and timber processing industries, specifically in regard to enamel erosion (0.61 and 0.55 points) and multiple enamel cracks (0.79 and 0.76 points). Among agriculture workers, significantly lower values were noted (0.18 and 0.16 points, respectively). It should be noted that such disorders as fluorosis and dental hypoplasia did not show the influence of seniority on the frequency of occurrence: the correlation coefficients in respondents employed in the machine-building and timber processing industries were 0.16 points and 0.17 points, respectively; 0.15 points and 0.11 points. For almost all non-carious tooth defects, low correlation coefficients of work experience were observed in the agricultural population.

CONCLUSION

Thus, the analysis of the regional features in the incidence of non-carious tooth defects in the residents of the Penza region showed the following. The region is endemic for fluorosis, which is why there are high prevalence rates of fluorosis and hypoplasia of teeth. At the same time, the influence of industrial environment on the incidence of various types of non-carious tooth defects in the workers of machine-building and timber processing industries in the Penza region was established.

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IMPLANT TREATMENT IN PATIENTS WITH SEVERE TOOTH WEAR ON THE OCCLUSAL SURFACE

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ABSTRACT — Examination along with implant treatment was carried out in 21 patients with severe tooth wear on the occlusal surface. This disorder features a variety of clinical symptoms including changes in the facial features and the lower jaw movement amplitude, disturbed canine and incisor guidance, supercontacts, asynchronous operation of the masticatory muscles, etc. We have proposed a treatment algorithm aimed at eliminating these issues.
The temporary non-removable dentures allowed creating occlusal marks (Stage 1) followed with their transfer onto permanent orthopedic structure (Stage 2) using implants. The complexity of the approach to treating this disease, as well as the effectiveness of our method has been confirmed by clinical data and the outcomes obtained through an instrumental study involving electromyography and electronic axiography.
The extra additional research methods employed, such as electromyography and electronic axiography, allowed to assess the activity of masticatory muscles and the lower jaw articulation, thus to ensure occlusal-articulation interaction when restoring the lower facial height.

KEYWORDS — increased abrasion, dentures, electromyography, electronic axiography.

INTRODUCTION

Increased abrasion of the hard dental tissue (IDA) on the occlusal surface entails a number of anatomical, morphological, aesthetic and functional disorders. The prevalence of the issue ranges from 6% to 14.5% [1–3], the most common signs being a decrease in the size of the crowns affecting all teeth, dentin hyperesthesia, supercontacts, alterations affecting the shape of the dental arches, as well as a shortened lower third of the face.

Significant dental abrasion on the occlusal surface involves a change in the lower jaw movement amplitude, which is due to a decrease in the inter-occlusal height. The patient may fail to close the teeth in the previous position; there is also a frequent loss of the function guiding the canines and incisors, whereas the patient's own perception of the bite changes. Increased dental abrasion is an etiological factor behind the temporomandibular joint (TMJ) dysfunction [4–6], it is often accompanied by a partial adentia, which poses certain difficulties for implantation [7].

Considering the negative impact of occlusion-articulation changes due to increased dental abrasion [8], and the risk of changes in bite forces after the implant treatment, restoring the structure on the occlusal surface of dentures appears to play an important role [9, 10]. Lack of occlusion reference marks complicates carrying out rehabilitation measures up to their fullest extent [11–15]. In this regard, the necessary steps include proper remodeling of the occlusion, restoring the lower face height, and monitoring the functioning of the newly designed orthopedic structures, both shortly after setting the dentures, and long-term.

Aim of the study:
to improve the effectiveness of orthopedic treatment with implants in patients with increased dental abrasion on the occlusal surface.

MATERIALS AND METHODS

We examined 26 patients aged 35 to 53, among them 16 males and 10 females, who had increased dental abrasion on the occlusal surface, complicated by partial adentia. 93 implants (Semados) were installed in the patients. The inclusion criteria for the study and further implant treatment were patients with tooth defects of Class III and IV according to Kennedy classification. The exclusion criteria were degenerative changes in the TMJ structural elements.

The data involving the respective medical records, the patients' complaints, as well as the data obtained through clinical and instrumental examinations were analyzed. There were also examinations caried out such as the upper and lower jaw model analysis (Stratos 300 articulator), intraoral radiographs of teeth (Kodak 2000), and computed tomography imaging (Kodak 9000). The electronic axiography was performed with Arcus Digma II (Kavo Germany), electromyography of masticatory muscles — with Synapsis (Neurotech Russia). The statistical data processing for the obtained outcomes was done on the SPSS 25

software package, with the arithmetic mean (M) and the mean square deviation (SD) calculated.

The differences between the samples were evaluated employing the Student's t-test as well as the Mann-Whitney-Wilcoxon U-test, while between the results in the same sample through treatment — with the Student's paired t-test and the Wilcoxon paired test. The results were considered different at a significance value set at $p<0.05$.

RESULTS AND DISCUSSION

The patients were found to have a 8±1.24 mm decrease in the lower face interocclusal height; deepening nasolabial and chin folds; a deformed dentition; balancing supercontacts of molars in the lateral occlusions (right and left); canine disocclusion; disturbed incisor and canine guidance, as well as a partial adentia.

Since increased dental abrasion on the occlusal surface was accompanied by a significant decrease in the gnathic height, the restorative treatment was carried out in stages. Initially, a special orthopedic preparation was done (9) in the form of occlusal splint-mouthguards, which the patients should use for 3±0.15 months, and which allowed adjusting the TMJ elements prior to the implantation. 2 patients reported pain in the TMJ area, so they were further treated with the mouthguard, and the implantation had to be postponed.

24 patients were found to reveal no complications, and they had temporary fixed dentures (Stage 1) made for them at a set occlusal height according to our manufacturing method (Invention Patent RU 2453290 C1), which ensures functions like chewing, stabilized occlusion, phonetics and aesthetics. The point of the method implies the following: temporary fixed bridge-like dentures were modeled from wax; occlusion analysis was performed in the Stratos 300 articulator; the wax was replaced with a bis-acrylic material (Protemp 3 Garant or Luxatemp). The specific feature about these structures was that on the internal occlusal surface of the bridge-like prosthesis a fiberglass tape (Fiber Splint, Ribbond, and others) was adjusted in order to strengthen the frame of the entire structure. In case of IDA, the thickness of the chewing surface is usually sufficient enough. The manufactured structures were fixed temporarily, after which the occlusion was evaluated with instrumental control performed through electronic axiography and electromyography of the masticatory muscles. The results obtained were as follows (see Table 1, Stage 1).

During the dental implantation, the temporary bridges in the implantation area were removed, with the implants installed, and then the temporary fixation was repeated for the osseointegration period. At the end of the osseointegration, permanent dentures were installed.

In order to ensure the predictability for functioning of the orthopedic structures aimed at long-term positive outcomes, repeated control of the occlusal balance in the arches was carried out, with the following results obtained (Table 1, Stage 2).

The values proposed by V.P. Tlustenko et. al. were accepted as the criteria of the norm [16].

At the Stage 1, the masticatory muscle electromyographic examination testing the maximum jaw compression revealed a significant decrease in the biopotentials amplitude and an approximation to the normal values. The average amplitude indicators of the temporal muscle biopotentials were: 534.6±54.2 μV — on the right and 537.8±54.7 μV — on the left ($p<0.05$ compared to the norm); for the masseter muscles: 598.4±59.2 μV — on the right and 583.7±57.8 μV — on the left ($p<0.01$ compared to the norm). The symmetry factor (Sf), which reflects the activity symmetry both between the two similar muscles on both sides, and between the masticatory and temporal muscles on one side, went up reaching the following values: for the like-named muscles — 0.8±0.1 (temporal muscles) and 0.9±0.1 (masseter muscles, $p=0.002$); for the synergist muscles — 0.8±0.1 (the temporal and the masseter muscles on the right) and 0.8±0.1 (the temporal and the masseter muscles on the left), yet in all cases the value was significantly different from the norm ($p<0.01$). Given that, improving the occlusal relationship, the interalveolar height through temporary structures will allow arriving at significantly optimized activity of the masticatory muscles already at the preliminary treatment stage.

According to the EMG results, 1 month after the permanent prosthetics were completed (Stage 2), the indicators approached the normal values and, regarding the temporal muscles, were as follows: the average biopotential amplitudes — 523.6±52.8 μV (on the right, $p=0.056$ compared to the norm) and 514.9±52.9 μV (on the left, $p=0.226$ compared to the norm), the symmetry factor – 0.9±0.1 ($p<0.001$ compared to Stage 1, and $p=0.002$ – compared to the norm); for the masseter muscles: 551.2±54.2 μV (on the right, $p=0.280$ compared to the norm) and 541.1±53.9 μV (on the left, $p=0.415$ compared to the norm), the symmetry factor was 1.0±0.1. We believe that a slight excess in the indicators, if compared to the normal values, in certain patients, which was observed following the completion of the orthopedic treatment, could be accounted for by the remaining parafunctional activity of the masticatory muscles, which, as the modern ideas hold it, cannot be completely stopped.

Table 1. *EMG indices in patients at Stage 1 and Stage 2 through the orthopedic treatment, as compared with the normal values obtained through a functional test — maximum jaw compression in case of normal occlusion*

EMG indices	Td A avg. (μV)	Ts A avg. (μV)	Md A avg. (μV)	Ms A avg. (μV)	Sf Td-Ts	Sf Md-Ms	Sf Td-Md	Sf Ts-Ms
Stage 1	534.6±54.2	537.8±54.7	598.4±59.2	583.7±57.8	0.8±0.1	0.9±0.1	0.8±0.1	0.8±0.1
Stage 2	523.6±52.8	514.9±52.9	551.2±54.2	541.1±53.9	0.9±0.1	1.0±0.1	0.9±0.1	0.9±0.1
Normal status	491.9±52.9	495.4±51.2	533.4±52.5	527.5±54.4	1.0±0.1	1.0±0.1	0.9±0.1	0.9±0.1
p Stage 1 – normal	0.012	0.012	<0.001	0.002	<0.001	0.002	0.002	0.002
p Stage 2 – normal	0.056	0.226	0.280	0.415	0.002	0.990	0.985	0.990

Note: *Td — right temporal muscle; Ts — left temporal muscle; Md — right masticatory muscle; Ms — left masticatory muscle; Sf — symmetry factor. The statistical significance is shown in terms of the deviation from the normal value through each of the stages. The difference between Stage 1 and Stage 2 in all cases — p<0.001*

The change in the closure of the arches caused by increased dental abrasion, has a direct link to disturbed articulation of the lower jaw, which can be diagnosed through electronic axiography (Table 2).

The graphical parameters featured a lack of symmetry, a decreased length of the trajectories (this indicative of articular head restricted mobility due to functional overloaded masticatory muscles through their parafunctions), emerging premature interdental contacts that block and change the lower jaw movement, disturbed coordination of the masticatory muscles and the asynchrony of their inclusion in activity. Prior to the treatment, the sagittal articular course angle exceeded the normal values, which could be due to a changed rotation-vs-progress component ratio related to opening the mouth, with the rotational component predominating.

At the temporary structure stage, the sagittal articular course angle decreased to 47.8±4.7° on the right and 48.3±4.1° on the left. The articular trajectories of the lower jaw movements appeared as smooth curves concaved downward with the following linear indicators: 11.7±1.8 mm — the right joint trajectory length, and 12.1±1.9 mm — the left one (p<0.001 compared to the norm). However, a number of axiograms revealed a discrepancy between the mouth-opening trajectory and the lower jaw movement trajectory at closing the mouth, whereas the discrepancy exceeded 0.5 mm.

Upon completion of orthopedic treatment, the angles of the sagittal articular course angles decreased on both sides, if compared to the initial values (p<0.001) and were 44.6±3.9° on the right and 45.4±4.3° on the left (p=0.210 and p=0.339 compared to the norm), whereas they also got to feature some relative symmetry of the right and left sides.

An examination of the Gothic angles revealed amplitude symmetry of the lower jaw interincisal point movements, both to the right and to the left. The lines were not observed to have any shortening in the trajectory length, while the trajectories themselves appeared smooth. The protrusion line divided the Gothic angle into two identical parts in 85% of cases.

The obtained features pointed at synchronous performance of the masticatory muscles, as well as the absence of clinically significant dysfunction of the TMJ.

CONCLUSION

Employing all the above-mentioned preparation stages and orthopedic treatment of IDA on the occlusal surface, the inter-occlusal height and the central ratio of the jaws were identified optimally in view of the data obtained through electromyography and electronic axiography. Individual movements of the lower jaw in three mutually perpendicular planes, as well as the lateral movement amplitudes within the physiologically normal value range, were arrived at. Restored trajectories of protrusion, laterotrusion, mediotrusion and lateroprotrusion further allow optimal sliding of the lower jaw articular head along the articular tubercle slope, whereas disturbance in these principles can affect the TMJ function.

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Table 2. *Angular and linear indicators of electronic axiography during the open-close- mouth test in patients at Stage 1 and Stage 2 of orthopedic treatment, if matched against normal values*

Indicator	Sagittal articular course angle (°)		Articular trajectory (mm)	
	right	left	right	left
Stage 1	47.8±4.7	48.3±4.1	11.7±1.8	12.1±1.9
Stage 2	44.6±3.9	45.4±4.3	12.6±2.0	12.7±2.8
Normal value	43.2±3.3	44.1±4.5	14.8±2.1	13.9±2.0
p Stage 1 – norm	0.001	0.003	<0.001	0.004
p Stage 2 – norm	0.210	0.339	0.001	0.112

Note: *The statistical significance is shown in terms of the deviation from the normal value through each of the stages. The difference between Stage 1 and Stage 2 in all cases — p<0.001*

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EVALUATING OPTICAL DENSITY OF ALVEOLAR BONE IN PATIENTS WITH DIABETES MELLITUS USING CONE-BEAM COMPUTED TOMOGRAPHY

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ABSTRACT — The study involved an analysis of optical density of the maxillary and mandibular bone tissue based on cone-beam computed tomography data. It has been shown that the structure and bone tissue density depend on the severity of diabetes mellitus and complications. The results of the study revealed a significant decrease in the bone tissue optical density at the tooth necks in people suffering from diabetes mellitus, whereas fewer changes were manifested at the middle third of the dental roots. Minor changes or even an increase in the optical density were observed at the dental root tips.

KEYWORDS — diabetes mellitus, dental computed tomography, alveolar bone tissue, optical density.

INTRODUCTION

The past decade has witnessed an increase in the number of diabetic patients in Russia by more than 1 million people, yet the true prevalence of the disease is 2–3 times as high [1].

Diabetes mellitus is a metabolic disease entailing a high level of complications [2–8]. The literature claims [9–11] that violated connections of protein and mineral components, as well as deteriorated trophic tissues in case of diabetes mellitus lead to delayed remodeling of bone tissue, which is the factor determining its density. Diabetic arthropathies represent a fairly common complication of diabetes mellitus and affect, as has been described in a number of publications, 58% of patients with Type I diabetes and 24% of patients with Type II diabetes [12]. The endocrine system has a complex effect on the musculoskeletal tissues structure and function, which suggests that either insufficient or excessive production of a particular hormone will lead, sooner or later, to pathological changes in bones, joints and muscles [13–15].

At the same time, apart from changes involving the quantitative features of bone tissue, it is important to investigate the qualitative parameters of bone, including in patients with diabetes mellitus. In particular, experiments on animals with insulin deficiency followed by a histomorphometric analysis of the obtained data indicate a decrease in the bone tissue development rate [16–18]. Besides, there was a decrease observed in the length of bone trabeculae, as well as in the periosteal and endocortical surfaces of the osteoid-covered cortical plate. At the same time, a decrease was observed in the number of osteoblasts, and a disturbance in their function, as well as an increase in the apoptosis rate [19–21].

Diabetes mellitus has been found to be a factor predisposing to the onset and progress of destructive periodontal diseases that lead to the loss of the dentogingival junction [25]. The current concept [24] holds it that in patients suffering from Type II diabetes mellitus, the primary role in the pathogenesis of inflammations affecting the alveolar part belongs to microangiopathies and acidosis due to high blood glucose content. Insulin insufficiency leads, on the one hand, to a decrease in the synthesis of collagen and alkaline phosphatase by osteoblasts, while the produced substances are involved in the development and mineralization of the intercellular matrix, and, on the other, to disturbances in the calcium absorption by the small intestine microvilli and its increased excretion from the body with urine. Hypocalcemia, in turn, stimulates the synthesis of parathyroid hormone, which results in thinning of the compact layer and in the bone tissue resorption.

Therefore, diabetes mellitus is accompanied with disturbed metabolic processes in the bone, disrupted functioning of its cellular elements and organic structure, which leads to violated biomechanical properties and, consequently, a greater risk of fractures. The

potentially involved mechanisms that at least offer a partial explanation to the causes behind bone deterioration in case of diabetes, include hyperglycemia and microangiopathy. Bone tissue fragility is an effect of insufficient exposure to insulin, and not a complication of diabetes mellitus [23]. This means that the mechanisms underlying the developing bone fragility are the same for both types of diabetes. Besides, it has no direct relation to diabetes and can occur long before its clinical manifestations.

Jaws bone tissues differ little from the rest of the skeleton in terms of their chemical composition and structure. However, the alveolar bone has internal restructuring processes going on faster than in other bones of the skeleton. Normally, the height of the alveolar ridge is maintained by the physiological balance between bone formation and resorption, which is regulated not only with systemic, yet also with local factors [26–29].

In modern dentistry, X-ray diagnostics methods make up an integral part of a comprehensive medical examination. Computed tomography (CT) is a relatively new method used to study the chewing system that allows obtaining high-resolution 3D images. Compared to 2D X-rays, 3D digital computed tomography can improve significantly diagnostics quality, including differential diagnostics, as well as reduce the risk of errors. All this is due to a higher resolution of the images obtained, as well as to the option, which allows a layer-by-layer examination on the computer screen [14, 22, 30].

Aim

The aim of this study was to identify optical density of the bone tissue in the alveolar parts of the upper and lower jaw in patients with diabetes mellitus using cone-beam computed tomography.

CLINICAL POOL AND STUDY METHODS

The comprehensive study involved 94 patients aged 31–75 (the median age being 53±5, with no gender factor taken into consideration), who were divided into 2 groups. Group 1 included 44 patients with diabetes mellitus (6 patients with Type I diabetes and 38 patients — with Type II diabetes), while Group 2 included 50 patients with no endocrine pathology. A clinical dental examination performed in order to carry out additional diagnostics, prior to orthopedic or surgical treatment, all patients were to undergo a cone-beam computed tomography examination.

The inclusion criteria employed through the study were: age — 18 and above; Type I or Type II diabetes mellitus in the history; lack of dentition issues or the presence of some small (1 to 3 teeth missing) and medium (4 to 6 teeth missing) defects through the dentition length. The inclusion criteria were: endocrine pathology accompanying diabetes mellitus; chronic diseases in the decompensation stage; oncological issues, as well as the following dental diseases identified: pathological tooth abrasion; large dentition defects, periodontitis. The exclusion criterion implied the patient's refusal to undergo the respective examinations.

The examination was performed on a Gendex-GXCB-500 cone-beam computed tomograph using the Icat Vision software. The optical density on the tomograms was estimated using a density window with a side of 3 mm. The measurements within the groups were carried out in the interdental septa of the teeth present in both jaws at the levels of their roots' mid- and top points, as well as the alveoli upper edges. Each of the measurements was performed three times. The average density value was calculated automatically by the software. The optical density was expressed in Hounsfield units (HU). The central trend and data dispersion were calculated with descriptive statistics methods. The quantitative parameters, depending on the distribution type, were presented as the mean value (M) and the mean square deviation (SD), or, when performing the assessment with nonparametric statistics, as the median (Xmed) and the interquartile QR range within the (LQ 25%÷UQ75%) range.

The analysis of the correspondence between the feature distribution type and the law of normal distribution was performed using the Shapiro-Wilk test. The critical level of difference significance when testing the statistical hypotheses was set at $p<0.05$. The nonparametric Mann-Whitney method was employed to analyze the differences between the subgroups identified subject to qualitative clinical and diagnostic features, with the Bonferroni correction factor used to estimate the Student's test values. The statistical processing of the obtained data was carried out using the Statistica 10 software.

STUDY OUTCOMES

As shown in Figures 1–6, the most significant changes affecting bone density could be seen at the tooth necks of the upper and lower jaws. Changes in bone density at the central part of the dental roots were less significant. Patients with this pathology featured an increase in the bone density at the tops of a number of dental roots.

A comparative analysis of the studied values revealed significant differences in the Gauss density in the group of patients, suffering from diabetes mellitus, due to a decrease in the bone tissue optical density, especially in the lower jaw (Tables 1–3).

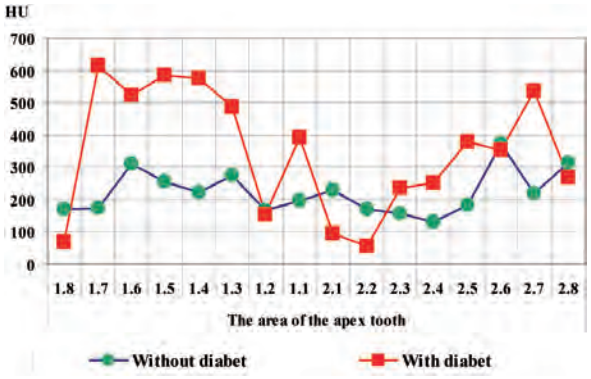

Fig. 1. Comparative characteristics of the maxillary bone tissue optical density by the Gauss density value at the tooth apices

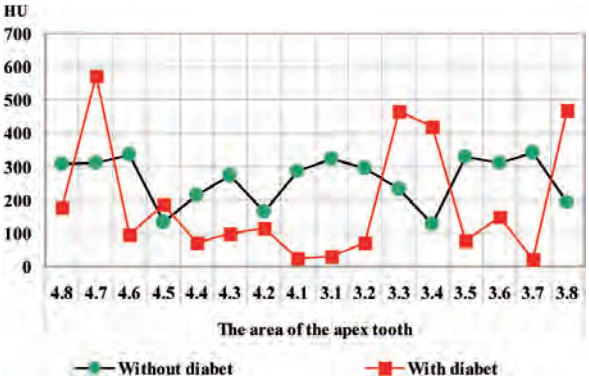

Fig. 2. Comparative characteristics of the mandibular bone tissue optical density by the Gauss den-sity value at the tooth apices

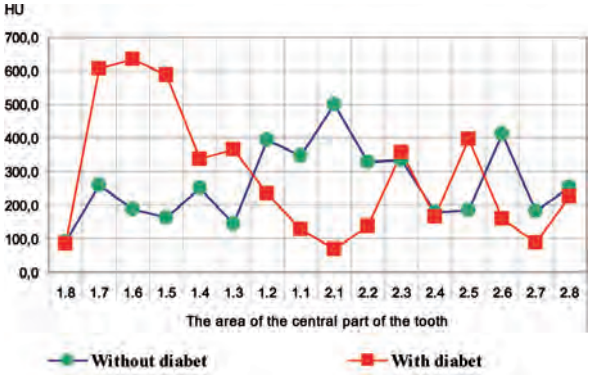

Fig. 3. Comparative characteristics of the maxillary bone tissue optical density by the Gauss density value at the central part of the dental roots

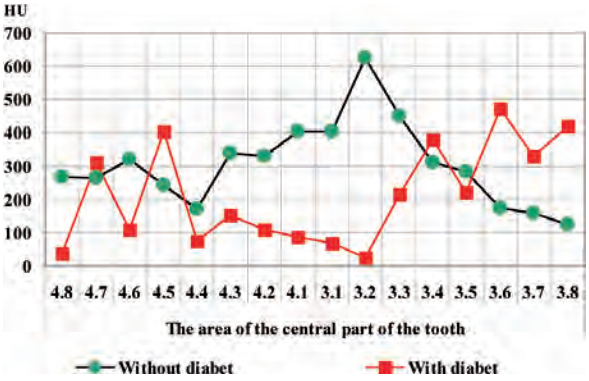

Fig. 4. Comparative characteristics of the mandibular bone tissue optical density by the Gauss density value at the central part of the dental roots

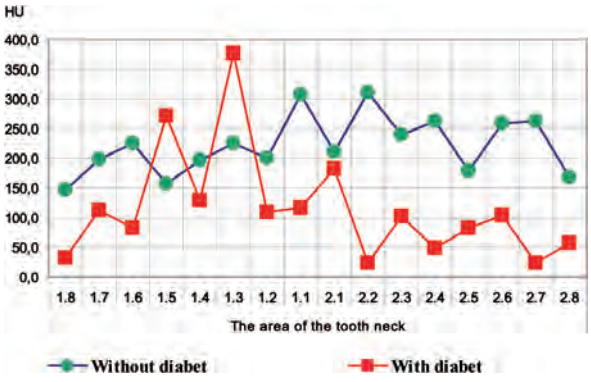

Fig. 5. Comparative characteristics of the maxillary bone tissue optical density by the Gauss density value at the tooth necks

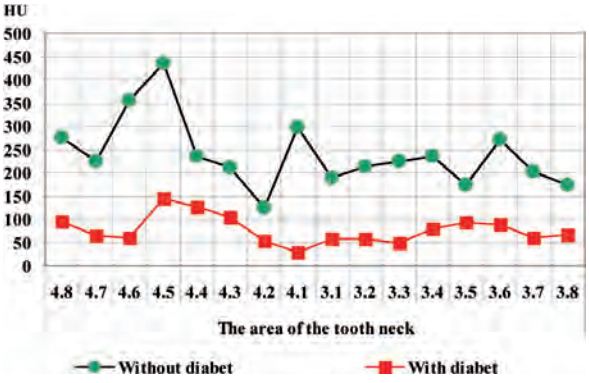

Fig. 6. Comparative characteristics of the mandibular bone tissue optical density by the Gauss density value at the tooth necks

A comparative analysis of optical density parametric data in the mandibular bone tissue in patients of the main and control groups revealed differences ($P<0.05$) at the dental root apices: 3.1, 4.1, 4.3, 4.4, 4.6 4.8; at the central part of the dental roots: 3.4, 3.3, 3.2, 3.1, 4.1, 4.2, 4.3, 4.4, 4.6; and almost at all the tooth necks, except tooth 3.8 (Table 4–6).

A nonparametric analysis helped reveal a range of differences in view of the median and the interquartile

Table 1. *Bone tissue optical density parametric data at the apices of the maxillary dental roots, HU*

Tooth no.	M±SD	m	M±SD	m	t	P
Patients without diabetes			Patients with diabetes			
1.8	235,2±107,3	87,4	166,7±47,7	32,9	1,626	0,0492
1.7	193,1±124,6	103,2	479,3±362,1	286,1	-3,807	0,0000
1.6	284,0±163,8	140,7	418,7±320,1	250,8	-1,517	0,0223
1.5	302,0±158,6	134,3	566,7±294,9	232,1	-3,426	0,0184
1.4	252,1±138,5	116,1	449,4±348,9	299,9	-2,427	0,0007
1.3	255,3±143,1	124,2	395,6±345,3	288,1	-2,067	0,0001
1.2	187,3±127,3	102,1	238,3±249,0	152,0	-0,932	0,0027
1.1	218,5±133,0	108,5	291,3±283,5	221,8	-1,230	0,0006
2.1	227,1±146,2	124,7	196,6±195,9	137,1	0,540	0,2075
2.2	239,9±191,5	145,7	151,2±75,8	51,4	1,357	0,0095
2.3	270,9±120,8	98,9	306,3±161,8	129,1	-0,850	0,1638
2.4	194,1±104,2	85,1	305,7±170,6	131,0	-2,517	0,0397
2.5	260,6±111,1	87,1	378,1±290,6	208,7	-1,926	0,0001
2.6	358,2±229,9	196,2	411,8±269,1	200,3	-0,580	0,4961
2.7	223,7±127,2	101,3	307,9±258,0	210,7	-1,320	0,0066
2.8	238,9±181,0	148,7	292,5±144,5	129,8	-0,653	0,6504

Note: *Values at P <0.05 are italicized*

Table 2. *Bone tissue optical density parametric data at the central part of the maxillary dental roots, HU*

Tooth no.	M±SD	m	M±SD	m	t	P
Patients without diabetes			Patients with diabetes			
1.8	237,0±95,1	68,8	133,6±40,4	30,8	2,781	0,0411
1.7	308,6±171,5	134,2	333,7±361,1	289,2	-0,281	0,0058
1.6	434,1±247,0	156,5	329,3±339,4	295,0	0,851	0,2688
1.5	302,8±106,9	87,2	506,6±282,3	228,4	-3,315	0,0002
1.4	447,4±184,1	148,3	364,8±211,3	163,5	0,987	0,5651
1.3	327,7±124,0	95,2	309,3±291,9	229,0	0,317	0,0001
1.2	434,0±222,2	189,0	189,5±193,5	145,8	3,203	0,6912
1.1	363,0±244,2	200,1	210,7±298,3	217,1	1,709	0,3630
2.1	473,6±256,1	227,1	181,1±329,1	193,5	2,982	0,2760
2.2	385,5±210,5	172,9	174,4±106,8	89,1	2,912	0,0480
2.3	475,4±265,8	188,7	314,0±242,2	209,5	2,029	0,4072
2.4	354,5±133,7	103,1	237,9±179,5	137,3	2,228	0,2124
2.5	470,4±224,4	193,8	304,0±244,2	195,8	0,649	0,1660
2.6	254,8±115,9	96,4	227,4±195,3	135,2	2,834	0,7479
2.7	304,5±170,4	143,9	132,4±54,8	44,8	2,712	0,0669
2.8	237,0±95,1	68,8	264,0±223,7	172,4	0,437	0,3879

Note: *Values at P <0.05 are italicized*

range (QR). Table 7 contains data describing the differences in the upper jaw, while the respective data for the lower jaw are presented in Table 8.

The obtained data point at significant changes affecting the optical density of bone tissue in patients with diabetes mellitus versus the control group due

Table 3. *Bone tissue optical density parametric data at the maxillary tooth necks, HU*

Tooth no.	M±SD	m	M±SD	m	t	P
Patients without diabetes			Patients with diabetes			
1.8	318,5±132,9	89,5	66,9±33,5	23,8	5,244	0,0010
1.7	349,7±177,2	140,8	129,7±72,0	56,8	2,978	0,0490
1.6	400,2±281,6	200,3	176,2±144,1	101,8	1,733	0,1989
1.5	315,3±106,7	85,2	250,3±149,2	125,1	1,370	0,2010
1.4	433,5±184,1	143,3	145,2±67,6	53,4	3,753	0,0339
1.3	350,5±144,2	121,5	174,9±179,4	154,3	3,323	0,3250
1.2	391,5±163,7	131,7	156,0±166,3	98,2	4,077	0,8640
1.1	435,8±220,7	174,9	148,5±152,3	92,2	3,896	0,2350
2.1	430,6±235,4	134,7	130,9±107,1	93,0	5,472	0,4783
2.2	486,7±197,9	172,0	98,3±79,5	53,6	5,748	0,0105
2.3	426,4±463,7	245,7	129,4±118,5	85,5	3,655	0,0260
2.4	488,7±333,3	192,7	115,5±76,0	49,6	5,228	0,0077
2.5	389,5±236,8	146,7	173,8±174,9	111,9	3,018	0,5800
2.6	423,3±165,3	134,1	144,7±109,2	80,1	4,780	0,2184
2.7	281,2±195,6	153,8	101,0±59,6	37,3	2,217	0,0144
2.8	345,1±126,9	96,9	93,3±41,6	29,2	5,081	0,0113

Note: *Values at P <0.05 are italicized*

Table 4. *Bone tissue optical density parametric data at the apices of the mandibular dental roots, HU*

Tooth no.	M±SD	m	M±SD	m	t	P
Patients without diabetes			Patients with diabetes			
3.1	267,7±166,9	138,4	92,3±60,1	40,3	3,088	0,003312
3.2	253,3±195,4	149,4	125,2±90,1	71,4	1,914	0,061267
3.3	265,2±151,2	121,0	285,5±308,0	251,6	-0,317	0,752303
3.4	231,1±128,6	96,0	288,7±255,2	199,8	-1,030	0,307934
3.5	268,9±181,4	154,0	426,4±144,7	96,7	-1,855	0,071230
3.6	265,4±164,7	138,3	264,3±291,9	185,9	0,012	0,990407
3.7	277,1±190,3	163,5	130,8±68,8	45,4	1,681	0,102474
3.8	235,2±151,4	120,2	395,6±305,9	249,7	-1,730	0,096501
4.8	385,0±171,8	146,9	127,7±115,8	93,0	3,482	0,001461
4.7	304,6±198,0	168,3	501,2±371,6	293,8	-1,788	0,083267
4.6	474,9±217,5	180,5	139,7±142,7	98,9	3,526	0,001728
4.5	215,7±94,4	75,0	289,3±166,8	130,1	-1,566	0,125471
4.4	283,4±129,6	109,9	123,0±64,5	46,2	3,778	0,000428
4.3	340,3±187,5	152,9	165,6±176,5	113,9	2,888	0,005603
4.2	181,4±113,4	89,3	115,4±70,3	57,6	1,672	0,100668
4.1	262,7±160,4	131,3	82,3±58,3	42,5	3,304	0,001785

Note: *Values at P <0.05 are italicized*

to the results of both parametric and nonparametric analysis.

RESULTS AND DISCUSSION

The analysis of the bone tissue optical density results in patients with diabetes mellitus, when com-

Table 5. *Bone tissue optical density parametric data at the central part of the mandibular dental roots, HU*

Tooth no.	M±SD	m	M±SD	m	t	P
Patients without diabetes			Patients with diabetes			
3.1	487,7±274,1	221,1	135,9±82,0	59,4	3,787	0,000417
3.2	453,1±321,6	285,2	101,7±49,3	33,3	3,246	0,002090
3.3	472,9±237,2	202,0	207,0±256,6	170,9	3,282	0,001808
3.4	412,7±209,5	178,2	212,7±214,9	178,0	2,700	0,009430
3.5	346,0±203,9	164,9	195,2±202,4	160,6	1,551	0,129072
3.6	330,3±135,2	95,6	323,6±241,6	211,5	0,093	0,926849
3.7	351,2±157,3	123,7	302,5±329,3	250,0	0,398	0,692992
3.8	249,8±102,3	79,5	348,5±307,5	258,2	-1,292	0,208338
4.8	422,6±187,3	156,0	238,4±203,7	139,0	2,002	0,049020
4.7	383,1±182,5	137,6	365,2±308,8	224,2	0,183	0,856131
4.6	414,3±222,5	164,6	100,5±64,8	52,8	3,368	0,002551
4.5	347,2±201,0	149,7	314,8±272,6	219,5	0,346	0,731023
4.4	434,8±169,7	132,3	111,7±81,9	55,4	5,823	0,000000
4.3	484,4±266,2	192,2	161,0±172,0	117,0	3,970	0,000218
4.2	396,9±231,3	176,2	114,6±83,3	62,0	3,590	0,000742
4.1	472,0±307,2	243,9	98,3±56,9	51,3	3,608	0,000722

Note: *Values at P <0.05 are italicized.*

Table 6. *Bone tissue optical density parametric data at the mandibular tooth necks, HU*

Tooth no.	M±SD	m	M±SD	m	t	P
Patients without diabetes			Patients with diabetes			
3.1	344,1±178,8	119,2	86,2±42,9	34,3	4,268	0,000090
3.2	349,3±202,3	139,3	94,7±54,2	43,3	3,723	0,000492
3.3	360,5±186,1	149,7	98,1±62,2	44,7	4,587	0,000027
3.4	314,5±173,0	135,5	141,9±208,1	120,0	2,728	0,008760
3.5	357,7±168,5	124,4	139,8±95,0	72,6	2,809	0,007734
3.6	348,4±166,8	143,0	153,9±58,7	44,7	2,992	0,006002
3.7	337,4±160,2	132,9	144,0±84,0	59,7	2,853	0,007418
3.8	239,6±109,3	85,5	154,0±142,8	94,0	1,583	0,125902
4.8	355,4±180,8	147,9	91,7±50,5	41,0	3,506	0,001372
4.7	369,4±254,6	166,9	111,4±87,3	61,7	2,217	0,034075
4.6	435,0±226,4	189,3	96,9±45,4	37,6	3,876	0,000681
4.5	496,7±248,8	202,8	155,0±88,6	76,0	3,014	0,004569
4.4	469,9±425,6	228,1	115,5±88,4	70,5	2,601	0,012269
4.3	489,6±439,4	222,9	116,8±69,2	59,7	2,910	0,005275
4.2	298,3±147,5	105,3	77,7±37,0	32,1	4,428	0,000050
4.1	441,6±213,9	165,3	76,6±43,4	32,0	5,058	0,000006

Note: *Values at P <0.05 are italicized.*

pared to the control group, showed that the most significant changes can be observed at tooth necks. Less significant changes were observed at the middle third of the dental roots. Minor changes or even an increase in the bone optical density were to be seen at the dental root apices. This data is comparable with the data

Table 7. *Characteristics of bone tissue optical density nonparametric data for the upper jaw, HU*

Tooth no.	Xmed	by QR	Xmed	by QR
Patients without diabetes			Patients with diabetes	
In the area of the apex of the teeth				
1.8	268,0	130,5÷302,0	168,0	141,0÷211,0
1.7	143,0	90,0÷263,0	432,4	187,0÷802,0
1.6	266,0	124,0÷436,0	419,0	117,3÷641,0
1.5	291,0	167,0÷421,0	595,0	314,0÷901,0
1.4	224,0	145,0÷366,0	402,5	123,5÷698,0
1.3	222,5	118,5÷392,0	208,5	152,0÷640,0
1.2	155,0	100,0÷267,0	166,0	98,0÷253,0
1.1	243,5	86,5÷283,0	169,5	83,0÷475,0
2.2	165,5	115,0÷285,5	148,0	115,0÷172,0
2.4	167,5	127,0÷257,5	324,0	155,0÷407,0
2.5	257,0	173,0÷356,5	245,0	128,5÷507,5
2.7	209,0	109,0÷329,0	184,0	81,0÷617,0
In the area of the central part of the roots of the teeth				
1.8	229,0	177,0÷266,5	142,0	88,0÷172,0
1.7	260,5	152,0÷412,0	190,0	47,0÷654,0
1.5	316,0	222,0÷385,0	498,0	238,0÷824,0
1.3	301,0	264,0÷407,5	206,0	77,0÷444,0
2.2	365,5	213,0÷541,0	198,0	86,0÷223,0
In the area of the necks of the teeth				
1.8	299,0	234,0÷380,0	64,0	45,0÷77,0
1.7	308,5	245,0÷444,0	147,0	66,0÷179,0
1.4	421,0	329,0÷526,0	157,5	76,0÷205,0
2.2	504,0	321,0÷631,0	72,0	56,0÷79,0
2.3	293,0	221,0÷461,0	83,0	50,0÷151,0
2.4	461,0	326,0÷589,0	95,0	71,0÷120,0
2.7	229,0	98,0÷361,0	92,5	84,0÷108,0
2.8	309,0	278,0÷445,0	93,0	63,0÷120,0

available in the respective Russian literature (Bondarenko N. N., Balakhontseva E. V., 2012, Nikolayuk V. I., Kabanova A. A., Karpenko E. A., 2015, Chuev V. P. et al., 2017, Khaibullina R. R. et al., 2018).

The upper bone density in the jaw of patients with diabetes mellitus ranged from 151.2 ±75.8 to 566.7±294.9 Hu at the tooth apices; from 132.4±54.8 to 506.6±282.3 Hu — at the central part of the roots, while at the tooth necks it ranged from 66.9±33.5 to 250.3±149.2 Hu. As for the lower jaw bone density, it ranged from 82.3±58.3 Hu to 501.2±371.6 Hu.in at the tooth apices; from 98.3±56.9 Hu to 365.2±308.8 Hu — at the central part of the roots, and from 76.6±43.4 HU to 155.0±88.6 Hu at the tooth necks.

Thus, there is a direct relationship to be seen between changes affecting bone density at the tooth necks, especially in the molars and incisors, which is explained by deteriorating periodontal trophism, inflammatory issues and a slowdown in bone remodeling. Changes affecting the area at the tooth apices are less significant. The area at the lateral group of teeth featured an inverse relationship, and here the bone density in individuals with diabetes exceeded that in the control group, which can be explained by the effect of reparative processes and the nature of blood supply in this area.

CONCLUSION

1. The bone tissue status of the upper and lower jaw alveolar parts, as could be seen from cone-beam

Table 8. *Characteristics of bone tissue optical density nonparametric data for the lower jaw, HU*

Tooth no.	Xmed	by QR	Xmed	by QR
Patients without diabetes			Patients with diabetes	
	In the area of the apex of the teeth			
3.1	288,5	73,0÷397,0	81,0	56,0÷86,0
4.8	417,5	214,0÷522,5	89,0	34,0÷211,0
4.6	489,0	314,5÷652,0	82,5	62,0÷157,0
4.4	296,0	163,0÷379,0	97,5	84,0÷156,0
4.3	348,0	212,0÷487,0	112,5	62,0÷160,5
4.1	290,5	75,0÷362,0	66,0	47,0÷72,0
In the area of the central part of the roots of the teeth				
3.1	516,0	264,0÷667,0	127,0	91,0÷161,0
3.2	470,0	134,0÷758,0	96,0	85,0÷109,0
3.3	475,0	248,0÷700,0	89,0	78,0÷292,0
3.4	445,0	236,0÷548,0	93,5	56,0÷436,0
4.6	397,5	239,0÷559,5	84,5	51,0÷161,0
4.4	431,0	329,0÷500,0	83,0	65,0÷140,0
4.3	477,5	294,0÷632,0	106,0	55,0÷207,0
4.2	422,0	167,5÷496,0	107,0	51,0÷161,0
4.1	455,5	254,0÷658,5	64,0	57,0÷146,0
In the area of the necks of the teeth				
3.1	337,5	231,0÷421,0	77,0	51,0÷108,0
3.2	332,0	224,0÷437,5	82,0	68,0÷126,0
3.3	320,0	231,0÷456,0	73,0	62,0÷110,0
3.4	258,0	189,0÷424,0	77,0	36,0÷115,0
3.5	339,0	246,0÷419,0	115,0	82,0÷176,0
3.6	296,0	241,0÷512,0	139,0	116,0÷205,0
3.7	314,0	241,0÷444,0	118,5	109,0÷170,0
4.8	295,5	225,0÷501,5	79,0	50,0÷145,0
4.7	301,0	225,0÷450,5	90,0	56,0÷225,0
4.6	418,5	233,5÷588,0	116,0	60,0÷120,0
4.5	459,0	322,0÷758,0	201,0	81,0÷225,0
4.4	366,0	256,0÷491,0	99,5	39,0÷166,0
4.3	428,0	314,0÷525,5	86,5	71,5÷176,0
4.2	266,0	193,0÷318,5	95,0	46,0÷100,0
4.1	450,0	314,0÷612,5	67,0	53,0÷82,0

computed tomography, showed a significant difference between the control group and the patients with diabetes. In case of diabetes, changes in the bone tissue optical density affected both the upper and the lower jaw, and were symmetrical in nature. Certain specific features identified on the right and on left halves may be explained by some peculiarities in chewing.

2. Patients with diabetes had the most significant changes in the bone tissue at the tooth necks of the upper and lower jaw, while the changes implied a decrease in the optical density.

3. Changes in the bone tissue optical density at the apices as well as at the central part of the dental roots were less pronounced. A decrease in the bone optical density in the patients with diabetes mellitus could be observed at the anterior group of teeth, both in the upper and in the lower jaw. At the molars and premolars of the upper and lower jaws, in the central part of the roots as well as at the dental root apices, there was an increase in the bone optical density.

4. The study outcomes suggest that changes in optical density can be used as an evaluation criterion

for assessing the jaw bone tissue status in people with diabetes mellitus. It could be also viewed as an important sign of early detection and forecasting of the progression of periodontal tissue disease.

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CLINICAL COURSE OF PERIODONTAL DISEASES IN PATIENTS WITH POSTMENOPAUSAL OSTEOPOROSIS TREATED WITH BISPHOSPHONATES

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ABSTRACT — The aim of the study was to assess the state of the clinical course of periodontal diseases in patients with postmenopausal osteoporosis (OP), depending on the long-term oral administration of various groups of bisphosphonates (BP). The study included 120 women aged 55–65 years with postmenopausal OP for at least three years who took complex antiosteoporetic therapy, including BP in tablet form. The dental examination included an examination of the oral cavity, the study of the pH of the oral fluid, the hygienic state of the mouth (the "Florida Probe" system). According to the results of the study, it was revealed that prolonged treatment of BP in tablets can provoke the development of inflammatory reactions in periodontal tissues. This phenomenon is associated with a shift in the pH of saliva to the acidic side, at which its buffer properties change.

KEYWORDS — periodontal disease, pH of mixed saliva, "Florida Probe" system, osteoporosis, bisphosphonates.

INTRODUCTION

Osteoporosis (OP) is a group of metabolic diseases of the skeleton characterized by an imbalance in the balance of the bone remodeling cycle, which leads to a decrease in bone mass, a violation of microarchitectonics, followed by an increase in fragility and the development of fractures [10]. A large increase in this pathology was observed in postmenopausal women [10].

Many studies have been described in the literature indicating that a decrease in bone mineral density contributes to a decrease in the height of the interdental bone septum, and as a result, the dentoalveolar attachment. Therefore, postmenopausal OP is a risk factor for periodontal tissue diseases [3, 4].

The first-line drugs in the treatment of postmenopausal OP are bisphosphonates (BP), whose pharmacokinetics vary, which is associated with the characteristics of the chemical structure and reflects the degree of therapeutic doses [8, 11].

The most pronounced antiresorptive effect in comparison with other BP has intravenous administration of the substance, but at the same time the risk of developing osteonecrosis of the jaws increases [7]. Therefore, oral BP is currently the most commonly prescribed treatment in patients with postmenopausal OP [6]. They have low bioavailability, and also have a number of side effects-primarily on the condition of the upper gastrointestinal tract. [9].

Black D.M., Bauer D.C., Schwartz A.V., Cummings S.R., Rosen C.J. (2012) note that in 6-30% of cases, these drugs cause a deviation of the pH of the stomach in the acidic side [9].

At the same time, it is proved that low pH values of mixed saliva cause the development of dental diseases [9].

In 2007, A. P. Leus, associated changes in the buffer properties of the oral fluid with the values of acid-base equilibrium. When the pH drops to 6.4, the saliva becomes undersaturated with calcium and inorganic phosphate and turns into demineralizing, which subsequently causes the occurrence of caries of hard tooth tissues [5].

According to a number of authors, with a decrease in pH values, the binding property of saliva proteins with calcium increases, playing a role in the processes of demineralization. When this association is violated, calcium is deposited on the surface of the tooth, forming a supragingival tartar, which is formed by saturating the plaque with calcium phosphate crystals, which creates an obvious pathogenic factor for the development of periodontal diseases [1,2].

It was also found that the acidification of the pH of saliva secretion is the result of anaerobic processes, resulting in an increase in the activity of hydrolytic enzymes that break down carbohydrates and proteinases that ensure the adhesion of plaque and microorganisms to enamel. At the same time, the activity of acid-stable proteinases increases by 1.5 times, which is

accompanied by the appearance of periodontal tissue inflammation.

However, according to the available literature, there are not enough observations to study the development of dental diseases in patients with primary OP with long-term use of BP in tablet form. Therefore, the problem of the effect of prolonged antiosteoporetic therapy on the dentoalveolar system becomes urgent.

Aim:

to evaluate the clinical course of periodontal diseases in patients with postmenopausal osteoporosis, depending on long-term oral administration of various groups of bisphosphonates.

MATERIALS AND METHODS

The study was conducted at the Department of Dentistry of the Central State Medical Academy of the Russian Federation from 2017–2020. The study examines 120 patients with primary OP who were treated and monitored at a dispensary for 10 years in the rheumatology department of the Federal State Budgetary Institution "Polyclinic No. 1"

Inclusion criteria: Inclusion criteria: women aged 55 to 65 years with diagnosed postmenopausal OP, who have been treated with BP (per os) for at least three years.

Non-inclusion criteria: patients with surgical menopause, malignant neoplasms, diseases of the parenchymal organs in the decompensation stage, autoimmune diseases, glucocorticoid therapy, therapy with BP for less than three years, the presence of pathological erasability (II, III degrees) of hard tooth tissues in the oral cavity, complete adentia of the dentition, secondary adentia of the teeth without orthopedic correction.

The diagnosis of osteoporosis was established on the basis of X-ray densitometry performed on the Lunar device in accordance with the Federal Clinical Guidelines for the Diagnosis and Treatment of Osteoporosis (2014).

120 patients who were on complex antiosteoporetic therapy (per os), including calcium preparations (1000 mg per day) and vitamin D (800 IU daily), depending on the BP taken, were divided into two groups:

I — 51 women — alendronate (70 mg once a week);

II — 69 people — ibandronate (150 mg once a month);

The comparison group consisted of 20 female volunteers aged 55–65 years without osteoporosis with chronic generalized parodontitis.

All subjects signed informed written consents to participate in the study.

The dental status was assessed after detailed monitoring of screening questionnaires, in which the patients, answering questions in detail, covered their health status, taking various medications during their life, including BP, as well as the presence of complaints from diseases in the oral cavity from the beginning of antiresorptive therapy and after three years of treatment.

The clinical stage included an examination of the oral cavity, the study of the pH of the oral fluid, the hygienic state of the mouth (the "Florida Probe" system), as well as orthopantomography on the "Planmeca ProMax"device.

To indicate the acid-base balance, the mixed saliva was collected in the morning on an empty stomach in a sterile Petri dish ("Medpolymer" d=60 mm). Before taking the oral fluid, the mouth was rinsed with distilled water. Using a universal indicator paper ("Lach-Ner"), its pH was determined.

The diagnosis of inflammatory periodontal diseases was carried out using the "Florida Probe" system ("Florida Probe" Corporation, USA), which contains a probing device, an encoding device that generated data and, after processing them, the digital parameters were visualized on a computer screen.

Titanium dental probe with a movable tube-coupling with a diameter of 0.5 mm, provides a smooth study of clinical parameters with a constant pressure of 20 g/cm^2. The program automatically determined the following indicators in absolute values: the presence of plaque, the degree of bleeding gums and the depth of periodontal pockets (with high accuracy up to 0.2 mm).

The examination procedure did not cause the patient any sharp pain and discomfort. The received information was displayed on the monitor screen with audio accompaniment and in the printed version in the form of a graphic periodontal map (Fig. 1).

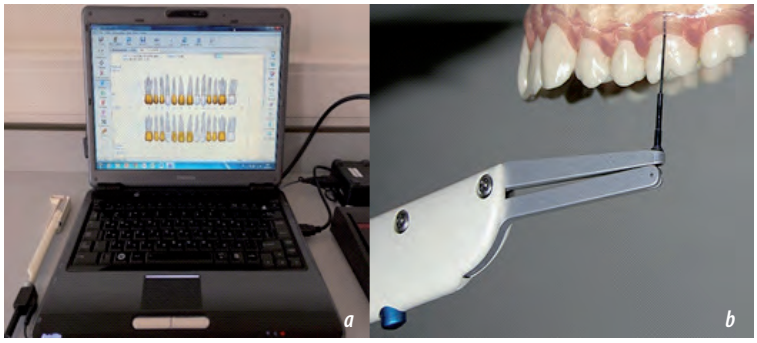

Fig. 1. *a — computer visualization of the periodontal map of the "Florida Probe" system, b — diagnostic probe*

The statistical analysis of the obtained data was calculated by the methods of variational statistics with the calculation of the Student's t-test and the degree of confidence (p) using a computer and the standard software package STATISTICA 10.0 (StatSoft, Inc., USA).

RESULTS

Monitoring of screening questionnaires revealed that the number of visits to the general dentist increased in patients with postmenopausal OP, regardless of the group and frequency of taking BP, after six months of treatment, which is associated with complaints of partial absence of teeth and periodic swelling of the gums. 93% of women noticed bleeding gums when eating solid food or while brushing their teeth, 73% — indicated the presence of recurrent caries and loss of fillings after six months of taking antiresorptive drugs.

It should be emphasized that all respondents rarely followed the standard rules for taking BF tablets (taking the drug on an empty stomach with a large amount (200 ml) of water, staying at least 1 hour in an upright position), which can be associated with the occurrence of complications from the gastrointestinal tract in the form of gastroesophageal reflux and the appearance of acidity in the stomach.

In all patients with a diagnosed OP, the pH of mixed saliva shifted to the acidic side, and the digital criteria depended on the frequency of taking BP. With weekly administration of alendronate, the acid-base balance parameter was the lowest (5,2±0,03), in comparison with the group receiving monthly therapy (5,9±0,03). Both coefficients significantly ($p<0.05$) differed from the control group, where the pH is within neutral values (7.4±0.04).

Examining the hygienic state of periodontal tissues, using the device "Florida Probe", in groups I (alendronate, 70 mg weekly) and II (ibandronate, 150 mg monthly), soft plaque and hard dental deposits were detected (75% — I, 71% — II). However, the data showed no significant differences between the groups and the control.

Analyzing the data of orthopantomography, all women of group I revealed a significant uneven loss of bone tissue of the interalveolar septa within ½–⅔ of the root length, which corresponded to a severe degree of periodontal disease. Mainly, the highest level of it was observed in the area of the lateral group of teeth of both jaws.

Studying the periodontograms obtained using the "Florida Probe" system, in women who took alendronate weekly (70 mg), the value of the degree of bleeding of the gums during probing was equal to 4.6±0.04. Assessing the depth of periodontal pockets, the highest indicator was noted in this group and corresponded to the level of 7.5±0.04 (Fig. 2). Both signs were statistically significantly higher than the values obtained in women without OP with chronic generalized parodontitis.

With a monthly intake of ibandronate (150 mg) all subjects of group II were diagnosed with chronic generalized parodontitis of moderate severity. On orthopantomograms, an uneven decrease in the height of the interalveolar septa by ½ the length of the roots in the area of all teeth was determined.

Examining the indicator of the degree of bleeding of the gums in women who were on monthly treatment, its average value was established (3.1±0.03). Against this background, the digital values of the depth of the clinical pocket were — 4.78±0.03. As an example, Fig. 3 shows a periodontal chart of patient B. 61 years old, who took ibandronate for more than 3 years.

DISCUSSION

Long-term use of complex antiosteoporetic therapy reduces bone resorption by suppressing the activity of osteoclasts, which leads to an increase in bone mineral density, but at the same time, long-term use of BF in tablet form has negative sides: it can provoke the development of an inflammatory reaction in periodontal tissues, despite systematic observation by a dentist.

This phenomenon is associated with a shift in the acid-base balance of saliva to the acidic side, at which its buffer properties change.

As a result, the progression of periodontal pathology was observed. We believe that statistically significant changes in the periodontal status of patients who took BF are also associated with an increase in the virulence of the periodontal flora in an acidic environment. All of the above correlates with the authors' data indicating the relationship between the development of dental diseases with a decrease in the pH of the mixed secret and a change in its protective functions [1, 2, 5]

It should be particularly noted that women who received weekly therapy with alendronate (70 mg) were in a more unfavorable situation compared to patients with monthly ibandronate (150 mg). According to our data, the degree of gum bleeding during probing was 1.5 times more pronounced and the depth of periodontal pockets was 1.6 times more pronounced (Fig. 4, 5).

CONCLUSION

According to the results of the study, the highest risk of an active course of inflammatory periodontal

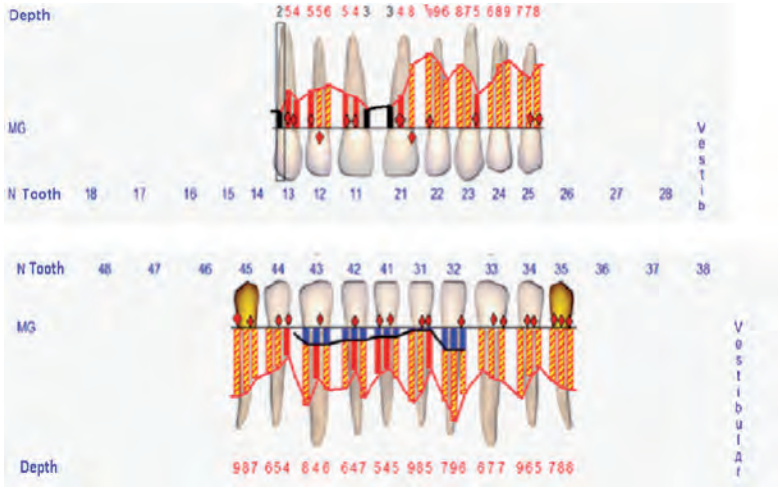

Fig. 2. *Periodontal chart of patient B. 61, treated with ibandronate for more than 3 years*

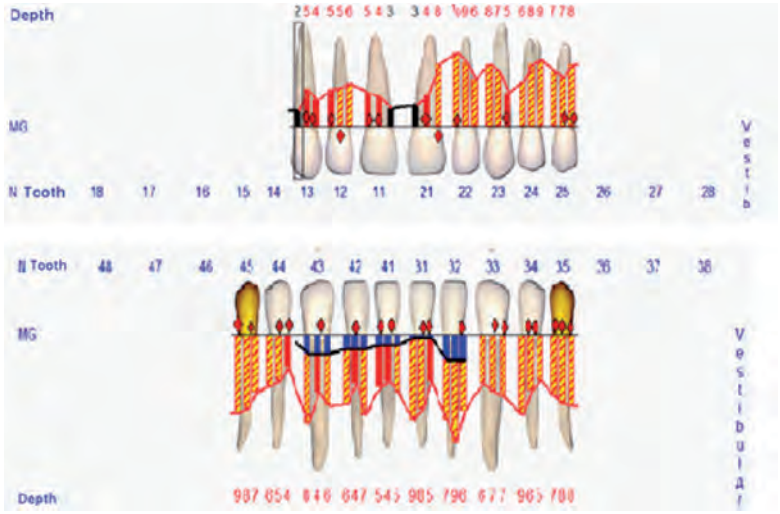

Fig. 3. *Periodontal chart of patient B. 61, treated with ibandronate for more than 3 years*

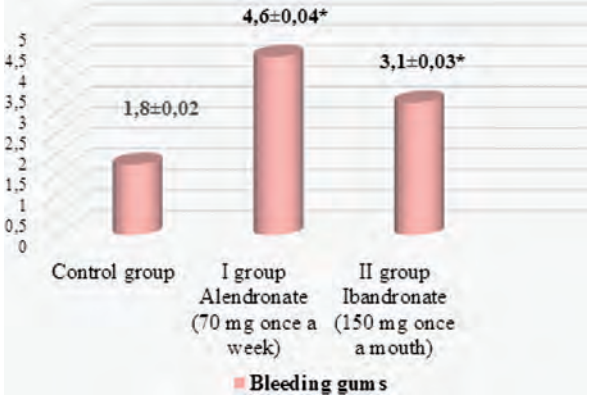

Fig. 4. *Comparative assessment of the values of gum bleeding in patients of the control, I and II groups*
(— the indicators have statistically significant differences with the data in patients with OP and normal bone mineral density), (p<0.05))*

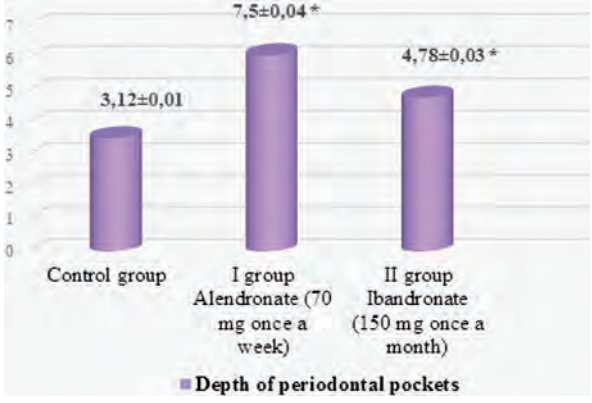

Fig. 5. *Comparative assessment of the depth of the periodontal pocket in patients of the control, I and II groups who took BP*
(— the indicators have statistically significant differences with the data in patients with OP and normal bone mineral density), (p<0.05))*

pathology is exposed to persons who take weekly tablet forms of alendronate 70 mg. It should be recommended that patients visit a dentist every three months in order to register the condition of periodontal tissues for preventive and therapeutic measures.

Important in the interdisciplinary interaction is the mandatory referral of women with postmenopausal OP, receiving BF, to the dentist to assess the monitoring of the state of the dental system.

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INFERIOR ALVEOLAR NERVE BLOCK BY INJECTION INTO THE PTERYGOMANDIBULAR SPACE USING GUIDING DEVICES: A SYSTEMATIC REVIEW

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ABSTRACT — The AIM of our research was to analyze and compare different inferior alveolar nerve blocking techniques and the effectiveness of various guiding devices. METHODS: A search was conducted on specialized databases for search and selection of works in which a guiding device for lower alveolar nerve block (IANB) was described. The propriated articles were evaluated and selected in 3 stages for final review based on predefined criteria, followed by a critical evaluation stage. As the research result - various types of IANB guide devices and the results of its using were recorded and analyzed.
RESULTS: The systematic review of devices for conducting IANB was done. The success of IANB can be achieved by adjusting the syringe with the anesthetic needle trajectory — it increased the probability of a successful hit in the area of the mandible foramen. Examples of devices in which the syringe is fixed at the moment when the tip of the needle is injected into the medial side of the branch of the lower alveolar nerve are considered. And also, the special techniques based on orientation on the soft tissues are described. However, when creating a device of this type, the following factors must be taken into account: the angle of the needle to the insertion point, the position of the insertion point relative to the anatomical landmarks (taking into account individual characteristics), and the insertion depth. Also, other IANB guiding blockade methods are considered: 3D navigation while local anesthetic injection. Thus, the patient's discomfort, the risk of nerve damage and the risk of unsuccessful mandible anesthesia could be minimized. CONCLUSION: The advantages and disadvantages of these anesthesia methods, the success rate, and patient comfort were analyzed. Prospects for further research in this area were identified.

KEYWORDS — anesthesia, inferior alveolar nerve blocking (IANB), guide device for anesthesia, pterygomandibular space.

INTRODUCTION

Nowadays patients pay great attention to the oral cavity health, particularly the teeth health (Ibrahim et al., 2017), (Yuan et al., 2020). But most of them still have a fear of dentist visiting. This is due the pain not only during treatment, but also during an anesthetic injection (Ushnitsky et al., 2018). According to Iwanaga et al., 2018 and Bhat et al., 2020, the most common mandible local anesthesia method is the inferior alveolar nerve blockage (IANB) (Iwanaga et al., 2018), (Bhat et al., 2020). A study by Rajvanshi, 2016 and Howait and Basunbul, 2019 showed a high efficiency of this method — 85% (Rajvanshi H., Ernest S., Hafsa Effendi H., Afridi S., Chhabra M., 2016), (Howait M., Basunbul, 2019). The authors point out that the anesthesia success is an accurate hit in the pterygoid — maxillary space. The most objective way to increase the IANB success is to determine the injection point taking into account the patient anatomical features (Farhangkhoee et al., 2012), (Petrikas A.Zh. et al, 2020). The injection point is located in the once center on the mucosa formed by the protrusion of the anterior inferior alveolar nerve branch edge (outside) and the medial part of the pterygoid muscle (inside) (Iwanaga et al., 2018). There are some intraoral landmarks described for IANB, which may vary depending on an inverted triangle of the individual's anatomy. It should be noted that according to Al-Moraissi et al., 2021, patients experience less pain during the blockade and it is more successful if using 27-gauge needles (Al-Moraissi et al., 2021).

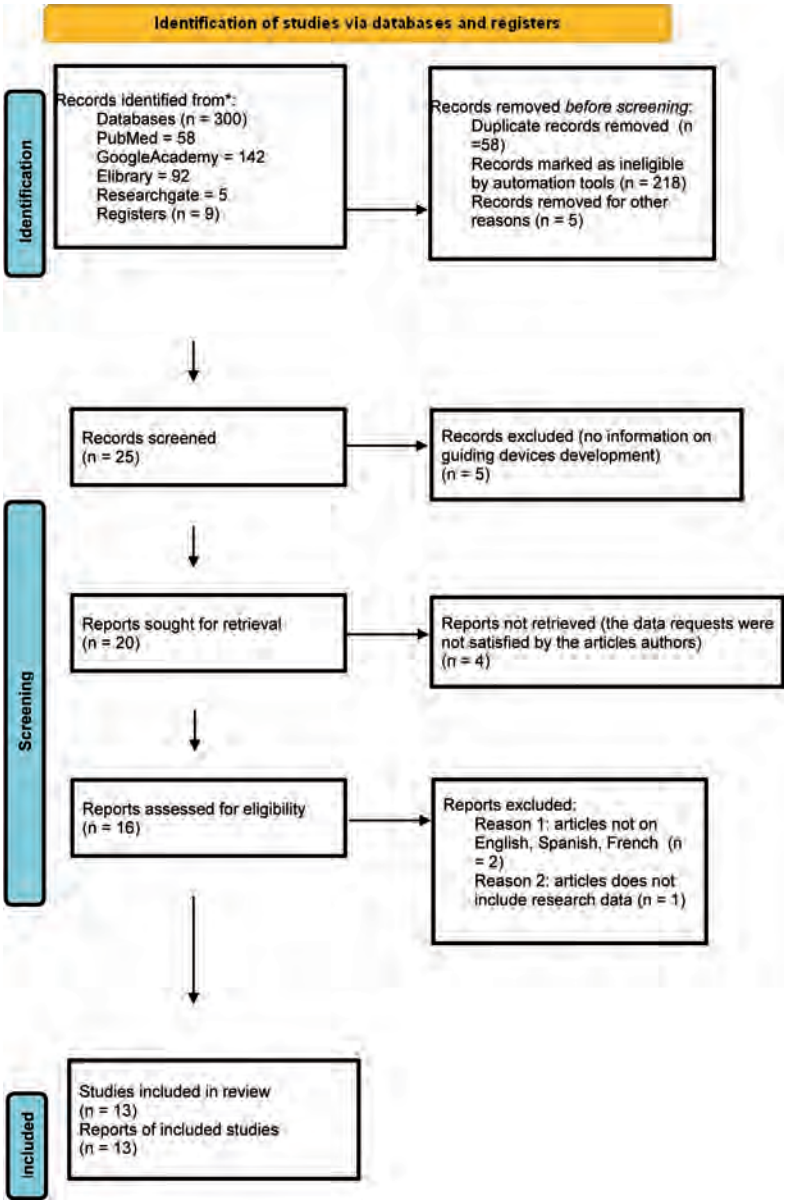
There are a number of adverse events during the IANB. When the needle is inserted into the medial pterygoid-mandibular fold, the anesthetic may spread to the oropharyngeal tissue and damage the internal pterygoid muscle, which could follow to the development of mandibular contracture (Rabinovich et al., 2018) (Kuzin et al., 2015). When the needle is inserted into the pterygomandibular fold or into its lateral edge, vascular damage, hematoma formation, anesthetic entry into the bloodstream, and the ischemic zones on the lower lip and chin skin appearance are possible (Alhindi et al., 2016). An allodynia, paresthesia, and dysesthesia are most commonly observed

(Ahmad, 2018). This accounts for the need to determine the precision of injection points and the need of a guiding device. (Farhangkhoee et al., 2012). There are some examples of the such devices implementation in the literature but there is no single solution that meets the request for a concise form, ease of use and manufacturer.

MATERIALS AND METHODS

A systematic review of several databases was carried out, with the studies selection (Scheme 1).

Scheme 1. *Methodology for selecting studies for meta-analysis. The original version is taken from the site: http://www.prisma-statement.org/).*



RESULTS AND DISCUSSION

The results obtained during the systematic review are presented in Table 1.

Besides, none of the listed devices has a full complete set of necessary functions:

— possibility of a single-uwse;

— adjustment for the patient's anatomy;

— secure attachment of the syringe;

— usage of both sides;

— additional maintenance of the patient's mouth sufficiently open for a dentist full-scale view;

— freeing of the one of the dentist's hands;

— adjustment for the speed and volume of the anesthetic supply

It should be noted that creating of such device should be based on previous studies and existing models. Also it is necessary to take into account such factors as: the needle angle to the injection point, the position of the injection point relative to anatomical landmarks (taking into account individual characteristics) and the depth of the injection. So the guiding devices for the IANB must meet most of the previously named points to ensure not only the success of the blockade, but also the comfort of the dentist and the patient.

CONCLUSION

This systematic review has shown that using various guiding devices increases the IANB success. Such devices can help avoid allodynia, prolonged anesthesia, paresthesia, dysesthesia and many other IANB complications. Also it was shown that there is no one point of view on the criteria for evaluating the advantages and disadvantages of guiding devices, so their further development is needed.

Table 1. *Description of existing devices for guided inferior alveolar nerve blockage (IANB)*

Author	Anesthesia type	Device description	Target injection point	Device locking points	Effectiveness	Advantages	Disadvantages	Notes
Egorov P.M., 1982 (Egorov P.M., 1982)	By Gow-Gates	Angulator for inferior alveolar nerve block	"The intermuscular triangle»	The mandible branch outer surface, at the level of the mandibular foramen	1 patient	The injection point was objectified	There is no significant simplification in the anesthesia procedure and there is no device fixing possibility	-
Jofré and Münzenmayer, 1998 (Jofré & Münzenmayer, 1998)	By Gow-Gates	Angulator for inferior alveolar nerve block	The point is distal to the second molar of the maxilla, slightly below the mesiopalate protrusion.	The ear part is inserted into the lower border of the tragus. The device itself is oriented in a plane from the lower border of the tragus through the corners of the mouth. After the plane is aligned, the syringe is placed in the device and the injection is made in the designated plane.	97,5% (39 patients at all)	The injection plane was objectified, and objective reference points were selected for all patients	There is no adjustment for: injection angle, depth and height.There is no a convenient way to hold the device.	One of the first objective out-of-mouth landmarks for the blockade was selected. It became possible to objectify the injection point.
Tiol A., 2001 (Tiol A., 2001)	IANB by Halsted	A device for targeting soft tissues. The methodology has been improved by Thangavelu et al., 2012. (Thangavelu et al., 2012)	On Tiol A., 2001 method (Tiol A., 2001), - deepening of the pterygoid ligament. On Thangavelu et al., 2012, method (Thangavelu et al., 2012) - from 6 to 8 mm above the midpoint between the upper and lower occlusal planes and the coronal notch and from 8 to 10 mm posteriorly from the anterior border of the branch	On Tiol A., 2001 method (Tiol A., 2001) – metal rod. It's the edge coincides with the recess at the pterygoid ligament, the guide is parallel to the mandibular occlusal plane (Tiol et al., 2010)	On Tiol A., 2001 method - (Tiol A., 2001) method - 94% (Tiol A., 2001) On Thangavelu et al., 2012, method (Thangavelu et al., 2012) – 95% (476 patient)	On Tiol A., 2001 method (Tiol A., 2001), - a convenient and safe method of anesthesia when targeting soft tissues. On Thangavelu et al., 2012, method (Thangavelu et al., 2012) – no additional devices for targeting the target point	The Tiol A., 2001 method (Tiol A., 2001): unsafety method and small evidence base. The Thangavelu et al. methood (Thangavelu et al., 2012): – increased risk of injury to the doctor. The absence of objective and fixed anatomical landmarks in both cases	The suitable method for teaching students to perform intraoral block anesthesia of the inferior alveolar nerve

Author	Anesthesia type	Device description	Target injection point	Device locking points	Effectiveness	Advantages	Disadvantages	Notes
Ushnitsky I.D., Chakhov A.A., 2020 (Ushnitsky, I. D. Chakhov, 2020)	IANB by Halsted	Assembly package for injection point position and the depth determining	It is possible to determine injections parameters during anesthesia individually for each patient	On the mandible branch posterior edge in the area of the greatest concavity and fix the measuring part of the set with the middle finger. Then the rod of this part is fixed in the oral cavity until it reaches the stop in the anterior edge of the lower jaw. According to the obtained measurements, the depth of immersion of the needle is determined. Then the 2nd part of the device is placed in the oral cavity until it stops at the mandible front edge and an injection is made along the guide arches.	1 patient	The ability to determine the depth of the needle injection individually for each patient.	A small amount of data is taken into account in the invention, the anesthesia itself is provided without objective guidelines, there is no possibility of simultaneous adjustment of all necessary parameters, the lack of reliable fixation of the device.	It can be used for mental anesthesia (Чахов А.А., 2019)
Zandi and Seyedzadeh Sabounchi, 2008 (Zandi & Seyedzadeh Sabounchi, 2008)	By Gow-Gates	Angulator for inferior alveolar nerve block	Distal to the maxillary second molar at the level of the mesiopalatyl plane.	The ear part is inserted into the lower border of the tragus. The device itself is oriented in a plane from the lower border of the tragus through the corners of the mouth. After the plane is aligned, the syringe is placed in the device and the injection is made in the designated plane.	93.3% (45 patients at all)	Ease of holding the device, objectified injection plane, selected objective reference points for all patients	There is no adjustment for: injection angle, depth and height	-
Caillieux N., 2009 (Caillieux N., 2009)	By Gow-Gates	The injection sagittal plane fixation along the mandible branch. The injection point selection in accordance to the syringe cylinder most lateral position in that plane.	The most lateral and highly located in the oral cavity, after the device placing.	The ends of the device clamp the mandibular branch between its anterior and posterior edges. The injection plane is selected according to the most lateral position of the syringe. For the injection, the highest possible point in the oral cavity is selected	No data	The easing navigation in the oral cavity	There is no regulation for the patient anatomical features, there is no regulation of the needle penetration depth into soft tissues, it is necessary to use doctor both hands, also devise is not stable, there is no regulation in height	There is a primary possibility of adjustment to the patient individual characteristics
Caillieux, 2017 (Caillieux, 2017)	By Gow-Gates	Angulator for inferior alveolar nerve block	From 7 to 25 mm (on average from 7 to 14 mm) from the mandible posterior edge at an angle of inclination from 53° to 62° (on average 32°)	Fixation behind the mandible posterior edge, with a maximum lag of 25 mm from that.	91,6%	A stable angle of inclination of the syringe to the injection site was created, the syringe was fixed, the calculations are based on the patients CT scans data objective analysis.	There is no regulation for the patient anatomical features, there is no regulation of the needle penetration depth into soft tissues, it is necessary to use doctor both hands, also devise is not stable, there is no regulation in height	A number of parameters were taken into account: the injection angle, the oral cavity parameters in the syringe passing plane, and the syringe was fixed in the appropriate position

Author	Anesthesia type	Device description	Target injection point	Device locking points	Effectiveness	Advantages	Disadvantages	Notes
Won and Kang, 2017 (Won & Kang, 2017)	IANB by Halsted	Superimposing a CT scan on the patient's oral cavity real photo	Direct orientation to the point of entry of the inferior alveolar nerve into the mandible (achieved by superimposing a CT scan on an intraoral photo)	-	No data	Выход обезболивания в AR пространство, разработан протокол для последующих исследований	There is no simplification in the anesthesia procedure.	This is the convenient method for teaching dental students the IANB method. But it is necessary to expose patients to excessive radiation exposure and s to train doctors to work in applications for processing DICOM images.
Chakhov A.A. Ushnitsky I.D., 2019 (A.A. Chakhov, Ushnitsky, 2019)	By Gow-Gates	Angulator for inferior alveolar nerve block	The lateral edge of the pterygoid-maxillary depression, immediately medial to the medial bundle of the temporal muscle tendon. The height of the injection point is determined by the location of the needle tip immediately below the medial-lingual (medial-palatal) maxillary second molar tubercle.	The outer ring is installed in the area of the outer surface of the condylar process. The direction of the injection is carried out in such a way that the target moves to the specified point.	No data	The additional reference point for anesthesia has been found, and a step has been taken to create a one-piece device with only 1 doctor's hand occupied.	There is no sufficient justification for the use of the technique, there is no way to objectify the injection point, there is no way to regulate the injection point in height	-
Jundt et al., 2020 (Jundt et al., 2020)	By Gow-Gates	Individual mouthguard for each patient, made according to the patient's CT scan data	From 3.8 to 10 mm above the occlusal plane, 3 mm medial and 6 mm higher from the point of entry of the inferior alveolar nerve	-	No data	Potentially high chance of success of anesthesia due to a personalized approach (the ability to take into account additional data)	Excessive radiation exposure of the patient, high cost and long-term manufacturing of the device	Ability to account for more data
Kojima et al., 2020 Kojima et al., 2020a (Kojima, Sendo, et al., 2020), (Kojima, Murouchi, et al., 2020)	IANB by Halsted	Ultrasound management of anesthesia	Standard for IANB	-	1 patient (Kojima, Sendo, et al., 2020) 28 patients (Kojima, Murouchi, et al., 2020)	Easy to perform in-hospital conducting anesthesia, designed for postoperative and rehabilitation	The method is difficult to implement in the conditions of outpatient admission, there is no objectification of the injection point, there is no significant simplification of the blockade, it is necessary to involve a specialist in ultrasound diagnostics.	-

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CLINICAL AND ANATOMICAL REQUIREMENTS AND INDICATIONS FOR USING A NOVEL MULTIFUNCTIONAL GNATIC DEVICE

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ABSTRACT — Knowledge of the clinical anatomy of the masticatory muscles contributes to the understanding of the etiopathogenetic and physiological aspects of the development of hypertonicity of the masticatory muscles. Hypertonicity of the masticatory muscles is a disease that leads to a number of other dental problems, such as abnormal abrasion, gum recession, trauma to the oral mucosa, wedge-shaped defects. We have developed and patented a pneumatic expander. It is feasible to treat hypertonicity of the masticatory muscles in a complex — myogymnastic exercises on the background of the use of the patented gnatic device. This paper presents clinical and anatomical requirements for the gnathic device as an uncoupling device in the treatment of both teeth and muscle relaxant.

KEYWORDS — hypertonicity, masticatory muscles, pneumatic trainer, myorelaxant.

INTRODUCTION

Hypertonicity of the masticatory muscles is often the cause of a number of pathological symptoms that lead not only to dental problems, but also other diseases (headache, migraine, stomalgia) [14]. Hypertonicity of the masticatory can arise under the influence of both general and local factors, while there is a two-way inverse relationship between the underlying factors and vice versa [3–8]. The impact on the group of masticatory muscles of the following factors: 1) General factors — impaired posture, neurogenic factors, autoimmune factors, traumatic factors; 2) Local factors — bite pathology, diseases of the temporomandibular joint, iatrogenic factor, bad habits. At the same time, there is an indirect inverse relationship between local and general factors [1]. The consequence of muscle hypertonia is the activation of anaerobic glycolysis, accompanied by the accumulation of lactic acid, which in turn irritates pain receptors, causing aggravation of pain symptoms [2, 11]. The emergence of a painful effect is a stressor agent, which leads to a more forced muscle spasm [10], against the background of the production of adrenaline. In parallel with this, there is a decrease in blood flow to the muscles, the formation of edema due to an increase in intercellular fluid and the appearance of painful muscle seals. The contracted muscle fibers lose their ability to function normally, which consists primarily of a partial loss of the chewing function [9] and the occurrence of other dental problems. Thus, a vicious circle is formed, which determines the duration of the course of the pathological process and its chronicity [13]. Based on this, we came to the conclusion that the place of the primary therapeutic effect in Hypertonicity of the masticatory should be the masticatory muscles.

The objective is to develop clinical and anatomical requirements for the use of a multifunctional gnathic device stimulating the masticatory muscles.

MATERIALS AND METHODS

Our proposed invention relates to medicine, namely to dentistry, and is intended for use in gnathic dentistry, as well as for separating the dentition during medical procedures in the oral cavity [12].

The pneumatic expander device has several effects, which is the clinical success. The functionality of the pneumatic expander-expander when creating an increased internal pressure in the bite block allows the device to be used as a fixer for the volume of mouth opening during various medical procedures in the oral cavity. The possibility of creating pressure in the cavity of the device makes it possible to controllably determine the degree of opening of the mouth, and, accordingly, the degree of stretching of the masticatory muscles, which is necessary in the treatment of patients with hypertonicity of the masticatory muscles. At the same time, the elasticity of the apparatus allows to use it as a stimulator counteracting muscle tissue atrophy. During the treatment and rehabilitation of patients, it is possible to use the apparatus in conjunction with a complex of myogymnastics to increase its effectiveness and reduce the duration of rehabilitation [12].

REQUIREMENTS AND INDICATIONS

In the available literature, we did not find the requirements for pneumatic trainers — rotary dilators, so we tried to formulate them based on their structure and function under normal conditions.

From our point of view, a pneumatic trainer should:

— provide therapeutic and prophylactic effects;
— transfer action to the chewing muscles;
— have a range of motion close to that of a healthy person;
— have a lightweight and durable design, adaptable to the anatomical parameters of the oral cavity;
— be bioinert and safe for the patient;
— carry out the expansion of the mouth by the amount of 40–50 mm;
— be elastic and durable, to maintain high intracavitary pressure;
— be able to cyclically dosed air injection to the required values and its release as needed, depending on the nature of the disease and the impact on it;
— provide adequate access to the dentition for therapeutic and prophylactic treatment by a doctor;
— possess simplicity and availability of activation for a doctor and a patient;
— exclude the possibility of slipping from the dentition;
— exclude the possibility of aspiration during manipulations;
— to be easily disinfected, cleaned and sterilized;
— have the possibility of individual anatomical parameterization;
— do not affect the observance of oral hygiene;
— be easily to withdraw in case of inflammation of the mucous membrane and injury to the gingival papillae;
— be mobile and independent of power sources and affordable for the mass consumer.

This device should only be used and prescribed by compliant patients making regular visits to a dentist.

The use of a pneumatic expander is indicated for the following diseases classified according to the ICD-10 code.

Many patients with TMJ do not go to the doctor, however, they note the presence of one or more of the following symptoms, which are indication for the use of a pneumatic expander.

— Muscle pain;
— Night grinding of teeth;
— Pathological abrasion of teeth, chips of orthopedic and therapeutic structures;

1. Dysfunctional conditions of the TMJ:	K07.6 – diseases of the temporomandibular joint (neuromuscular dysfunctional syndrome, occlusive-articulation syndrome, pain dysfunction syndrome) K07.62 – Habitual dislocation and subluxation of mandible K07.64 – Temporomandibular joint stiffness S03.0 – dislocation of the intra-articular meniscus S03.4 – Temporomandibular joint ligament sprain
2. Arthritis:	M00.VX – pyogenic arthritis of the TMJ M12.5X – traumatic arthropathy (acute traumatic arthritis) M05.VX – rheumatoid, rheumatic, infectious-allergic arthritis
3. Arthrosis:	M19.0X – primary arthrosis of other joints (TMJ) M24.6VX – joint ankylosis
4. Muscle damage:	F45.8 – Other somatoform disorders (bruxism, teeth grinding) M60.0 – Infectious myositis of the masticatory muscles M62.5 – Muscle wasting and wasting, not elsewhere classified M24.8.0 – muscle hypertonicity
5. Diseases of the nervous system:	G43 – Migraine G43.2 – Tension type headache K14.6 – Glossodyny

— Fatigue of the chewing and facial muscles;
— Pathological bite;
— Headaches, migraines, tinnitus and ringing in the ears caused by overexertion of the chewing muscles;
— Abnormal fixed postural reflexes;
— Restriction of mouth opening;
— Pain in the joint while eating, talking;
— Change of face configuration due to hyper; masticatory muscle atrophy.

CONTRAINDICATIONS

There are also absolute and relative contraindications for a gnatic device:

Absolute:

— Intolerance to the components of the pneumatic expander-retractor;
— Increased gag reflex;

Relative:

— Low level of compliance between parents and child;
— Acute respiratory diseases;
— Unstable psycho-emotional behavior of the child;
— Persistent contracture of the temporomandibular joint, with limited mouth opening less than 10 mm;

— Period of exacerbation of periodontal and temporomandibular joint diseases;
— Cheilitis and erosive and ulcerative diseases of the oral mucosa in the acute stage.

CONCLUSION

Thus, clinical effects of the use of a pneumatic expander are achieved by redistributing the occlusal load on the joint, which results in gradually normalizing the function of masticatory muscles. This may be seen as a solution to myology problems in oral and maxillofacial region.

The application of a multifunctional gnathic device can be complemented with exercises and relaxing techniques to improve the therapeutic effect on the masticatory muscles.

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