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# EDITORIAL

*Dear colleagues,*

Year 2020 is coming to an end and the 4th and last issue of our journal has been released. The passing year is an exceptional year for the whole planet but for all health care workers will be the toughest and most challenging one. Medical practitioners took the huge load and responsibility in the struggle with the pandemic of COVID-19. Practitioners and scientists has immediately responded with development of new treatment methods, new reliable tests and new vaccine for COVID 19.

Great number of papers has emerged presenting the studies how the virus affects different aspects of health. Apparently, never before in the medical history scientific studies have been conducted so intensively, nor there has ever been a disease where the recommendations on treatment have changed so often and rapidly as in this year. At the moment 10th version of the treatment scheme of COVID appeared. Right now doctors learn how more effectively combat this dangerous disease than in the beginning of the year. Nevertheless, this struggle is not over and the morbidity and mortality is increasing so far.

Moreover, the pandemic aggravates certain, already existing, medical problems, such as the antibiotic resistance and it creates new problems, globally disrupting hospital routine: planned elective surgeries have to be rescheduled or cancelled and chronic patients are neglected or scared to visit a doctor. These problems should be acknowledged and managed by specialists.

Anyway, life goes on and COVID-19 is not the only threat to health and we should not forget other important medical issues. Our journal has preserved its traditional structure but starting with the first issue (March 2020) the papers on COVID 19 are published.

This year has become a very important one for the journal team. We have to work harder to produce 4 releases. Furthermore, we see further potential for increasing the number of issues. This was only possible due to our editorial colleagues and our reviewers who found time and inspiration for working at the papers even in the toughest working and economic conditions. We want to thank our authors and colleagues for their great contribution and the collaboration in year 2020! Merry Christmas and Happy New Year! We wish you good health and success in Year 2021!

Yours,

*Editor-in-Chief*
***Dr. Georg Tyminski***

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# EDITORIAL

*Dear clinicians, research fellows, colleagues and friends!*

The prevalence of drug addiction and its asocial manifestations poses one of the acutest global problems. According to World Health Organization (2019), abuse of alcohol, drugs and other substances affecting the consciousness has reached the *epidemic* proportions in the beginning of 21st century.

On the global scope the consumption level of drugs remains high: in 2010 the illicit substances were at least once consumed by about million people or about 6% of the population of Earth aged from 15 to 64 years old. According to modern statistics in Russia about 670 thousands of active drug users (231,6 on 100 000), however, according to some experts this number can reach 2,6 million (or about 2% of the population). The correlation between registered drug addicts, episodic consumption of drugs and psychotropic substances and *hidden* addicts is described as 1:3:5. In the last decade the estimated mortality rates among drug users has increased tenfold. Female ratios among drug users has increased in 6,5 times, whereas deaths in adolescents — in 42 times. Independent specialists reported that in Russian cities only 5–7% of drug users are considered cured (withholding drugs more than one year).

The problem of drug addiction is getting younger and it is closely connected with the spread of HIV. The Russian Federation has the highest HIV prevalence, even higher than in African countries.

According to international statistics every year two-hundred-thousand people die from drug-related causes [World Drug Report, 2015]. Furthermore, life expectancy of a heroin addict is not more than 7 years.

The narcological situation to date is characterized by a decrease in consumption of opioid group while the growth in consumption of synthetic psychoactive substances among young people. According to the early warning system (the European Monitoring Centre for Drugs and Drug Addiction) in 2010 there were 41 new psychoactive substances, in 2011 — 49 and in 2012 — 73.

Synthetic cannabinoids were found as most common (39,3%), followed by synthetic cathinones (16,6%) and phenylethylamines (14,1%). As a consequence of the fact that only certain substances and not the classes of substances can be prohibited and are subject to law, manufacturers offer the consumers new compounds including the ones with unknown chemical structures.

New psychoactive substances are available and are in demand among drug users as they are supposed to be legal, cheap and with limited health risks. These subjects are not detected by standard immunological tests. For example, synthetic cannabinoids are not detected with a standard THC-test and they are not detected with ELISA-test on amphetamine. Only small part of psychoactive subjects is detectable with a standard methamphetamine test. The ring of piperazine, whose composition contains some substances, produces mixed results in the standard amphetamine test. More sophisticated methods are normally used to screen for psychoactive substances, mainly the method of Gas Chromatography Mass Spectrometry (GC-MS) and liquid chromatography–mass spectrometry (LC-MS/MS).

Therefore, an adequate test may be only performed either in a toxicological laboratory or a forensic institution.

With the help of certain biochemical markers specialists can evaluate susceptibility to drug addiction alongside with metabolites of drug substances

# EDITORIAL

and products of altered body metabolism. Assessment of such markers enables to make prognosis and set up preventive measures. This is targeted, in general, on struggling with drug addiction.

All biomarkers can be classified into 3 categories: drug substances and the products of their metabolism, markers of biochemical response on the syndrome model of addiction, genetic and epigenetic biomarkers associated with susceptibility to development of the addiction,

Among most perspective molecules there are neurotrophic factors (brain-derived neurotrophic factor, glial cell line-derived neurotrophic factors), other specific nerve tissue proteins (galanin, protein s100, neuron-specific enolase, alpha-synuclein), markers for neurotransmitter disorders, indicators of the in-flammation intensity, including markers of oxidative stress, indicators of vascular damage as well as genetic and epigenetic biomarkers, such as serum microRNA and DNA methylation.

In this issue you may read about new data on markers of endogen intoxication and oxidative stress in patients with problem drug use. Qualitative and quantitative evaluation of the markers of oxidative stress allows conducting laboratory monitoring of metabolic disorders in patients with opioid addiction, to control effectiveness of the therapy and rehabilitation and to predict possible complications and relapse.

*Executive Editor*
***Prof. Dmitry Domenyuk***

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# ANNIVERSARY GREETING

## *Congratulations on Prof. Valentin Kozlov's 80th Anniversary*

13 December 2020 Prof. Valentin I. Kozlov celebrated his 80th anniversary. Valentin Ivanovich Kozlov is MD, Professor, Outstanding Scientist of the Russian Federation and Director of Human Anatomy Department at Russian University of Peoples' Friendship.

Valentin Kozlov is an actual member of several Academies:

— International Academy of Higher Education;
— International Academy of Sciences in Munich;
— European Academy of Natural Sciences.

Valentin Kozlov studied at Military Medical Academy in Saint Petersburg and Pirogov Russian National Research Medical University in Moscow, which he graduated from in 1965. In 1973 he had established and headed the Laboratory for Microcirculary Studies there. From 1974 Valentin Kozlov was Director of Human Anatomy Department at the State Central Institute of Physical Culture. In 1977–1986 — hold the position of Deputy Director of Research at the Research Institute for Pediatric Physiology and at the same time joined there as Laboratory Director of Functional Morphology. From 1986 to 1996 he worked as Director of Medical-Biological Department at State Scientific Centre of Laser Medicine. Since 1996 Valentin Kozlov has been the Chief of Human Anatomy Department at the Russian University of Peoples' Friendship.

The fortunate combination of teaching and research has brought Valentin Kozlov respect and recognition at home and abroad. His research interests deal with investigation of structural and functional mechanisms of blood microcirculation and correction of their disorders. Another research subject is the interaction of laser radiation and biotissues. Valentin Ivanovich Kozlov has published more than 600 papers, among them more than 30 monographs and books. Valentin Kozlov has supervised 55 PhD and MD dissertations.

Today, at the day of his anniversary Valentin Kozlov continues to teach students at the Medical Institute of the Russian University of Peoples' Friendship. He writes books and articles, carries out research projects and helps young scientists.

Dear Professor, carry on like this! May your vitality and energy always replenish! We wish you new discoveries and creative inspiration, sound health and happiness!

***Editorial Board of the Journal***
***Archiv EuroMedica***
***European Academy of Natural Sciences***
***European Scientific Society***

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# OBITUARY

## Andrey V. Voronkov

### 22 January 1977—17 October 2020

Andrey Vladislavovich Voronkov died on 17 October 2020 aged only 44 years. Here is a tribute to our author and a wonderful person, an innovator, educator, MD and professor.

Andrey V. Voronkov graduated from Volgograd State Medical Academy in 2000. He started his professional life as assistant of the Pharmacological Department followed by research positions at the Research Institute of Pharmacology, in particular, at the Laboratory of Cardiovascular Medication. For a long time Andrey V. Voronkov worked as the Director of the Department of Pharmacology with a Clinical Pharmacology Course and the Vice Director of Pyatigorsk Medico-Pharmceutical Institute – the Branch of the Volgograd Medical University.

His major research interests included the development of substances with endothelial protective properties. These substances are aimed at correcting conditions of extreme physical and emotional load in high performance sport. His lifetime publication record lists more than 300 papers.

Andrey Vladislavovich Voronkov had a bright, intensive life. The Professor was valued and appreciated for his honesty, fairness, fearlessness and an ability to bring people together. Young specialists were encouraged and motivated with his professionalism, desire to discover and promote science. Under the guidance of Professor Voronkov 10 postgraduates successfully defended his PhD thesis. For his PhD students he was not only a strict and demanding mentor, but first of all an inspiration, a role model and just a friend you could rely on. Due to his experience and integrity Prof. Voronkov earned the respect and affection among colleagues.

Andrey V. Voronkov was a versatile and talented man. In addition to research and administrative activities he was a passionate athlete and the President of Professional Boxing Federation in Stavropol Region. Andrey Voronkov conducted numerous competitions and boxing matches attracting best professional boxers.

It is impossible to accept such untimely death, to imagine that in the corridor of the university we will never hear the Professor say *my young friends* to us. It has been our honor and privilege to work under the guidance of such a great scholar and a man. The memory of Andrey Voronkov will always be in our hearts. Finally, the team of post-graduates expresses heartfelt condolences to his family, friends and colleagues.

***With Deepest Grief***
***Post-Graduate Students***

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# THE MECHANISM OF BLOOD COAGULATION IN COVID-19 PATHOGENESIS

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**ABSTRACT** — One of the formidable complications of SARS-COV-2 infection leading to death is coagulopathy. The mechanisms of the development of this pathology at the present stage have not been studied, and clinical blood tests indicate that against the background of normal blood clotting indices, only the Ddi-D-dimer protein exceeds the norm many times over. The aim of the study was to study and analyze biochemical parameters in patients of Primorsky Region (Russia) infected with SARS-COV-2 against the background of concomitant vascular pathology and the development of DIC syndrome. The authors came to the conclusion about the existence of two mechanisms of circulatory disorders in the vessels of the microvasculature, associated with the violation of the integrity of the vascular wall and destruction of erythrocytes. Further research is needed to study the mechanisms of SARS-CoV-2 aggression, leading to thrombotic complications.

**KEYWORDS** — SARS-COV-2, COVID 19, coagulopathy, blood coagulation factors, prothrombin time, prothrombin index, Ddi - D-dimer, DIC syndrome.

## INTRODUCTION

The global COVID-19 pandemic represents a health emergency of unprecedented proportions. [11] Recent clinical data have shown that coronavirus disease 2019 (COVID-19) is associated with a significant risk of thrombotic complications ranging from microvascular thrombosis, venous thromboembolism, and strokes [6]. Importantly, thrombotic complications are markers for severe COVID-19 and may be associated with multiple organ failure and increased mortality. According to the literature, when infected with SARS-CoV-2, patients are predisposed to thrombotic complications in both the venous and arterial bloodstream due to excessive inflammation, platelet activation, endothelial dysfunction, and congestion leading to the development of disseminated intravascular coagulopathy (DIC syndrome).

Currently, there is no comprehensive understanding of the pathogenesis, treatment strategy and outcomes in patients with COVID-19 who develop venous or arterial thrombosis, especially in patients with preexisting thrombotic disease before the development of COVID-19 [3, 7]. The development of measures for the prevention and treatment of concomitant thrombotic disease during the COVID-19 pandemic is required. Al-Samkari H., Karp Leaf RS, Dzik WH, (2020) noted that elevated D-dimer levels in coronavirus disease 2019 (COVID-19) patients are inconsistent with observed bleeding rates, necessitating randomized trials to determine any potential prospects for enhanced effective anticoagulant prophylaxis in patients with COVID-19 [1].

Absence of general immunity in human to COVID-19 has contributed to a large number of infected patients around the world amid uncertainty about the treatment of complications arising from this viral disease [5, 9]. More than a million deaths in the world did not give an answer either about the causes of death, or about the features of the induction of pathomorphological changes by the SARS-CoV-2 virus [4, 12]. The high incidence of thromboembolic complications and high mortality suggest an important role for coagulopathy caused by COVID-19 [8].

Further research is needed to study the molecular mechanisms of coagulopathy in SARS-CoV-2 infection in order to develop conservative therapy to eliminate the pandemic. Although prophylactic drugs such as low molecular weight heparins (LMWH) are recommended by the International Society for Thrombosis and Hemostasis (ISTH) and the American Society of Hematology (ASH), their best effective dosage has not been determined at the present stage. These facts determined the direction of our research.

### *Aim of research*

To determine the features of the mechanisms of coagulopathy caused by COVID-19 when infected with SARS-CoV-2.

## MATERIAL AND METHODS

We studied the data of patients with laboratory-confirmed infection with SARS-CoV-2 and the development of the clinical picture of COVID-19, hospitalized in Primorsky Krai (Russian Far East) from March to July 2020. Data analysis was carried out in the dynamics of the disease for any thromboembolic complication, including venous thromboembolism (VTE), ischemic stroke, and acute coronary syndrome (ACS) / myocardial infarction (MI). The analysis of the clinical examination data from the recovered and patients with lethal outcomes was carried out in terms of prothrombin time (PT), international normalized ratio (INR), blood ferritin and platelet count, and the level of fibrinogen in the blood. The content of the D-dimer protein (Ddi), which is formed during the breakdown of fibrin and is the basis of blood clots, was also taken into account. The distribution of the clinical data material is shown in Table 1.

## RESULTS

Analysis of the data showed that among the patients infected with SARS-CoV-2, males prevailed, accounting for 54.8% of the number of patients included in the study group. Coagulopathy has been reported in nearly 95% of patients with severe COVID-19. Complications leading to death were severe disseminated intravascular coagulation (DIC syndrome). In the group with fatal outcomes, male patients predominated, accounting for 57% of the total number of deaths and about 56% of the number of COVID-19 hospitalized cases in men.

We noted that coagulopathy against the background of COVID-19 has shown that indicators such as PTT, PTI, platelet count, fibrinogen level in the blood of both men and women in the fatal group do not exceed framework of normal indicators. However, the measurement of the D-dimer levels showed that in deceased patients its content in all cases exceeds the normal values from 4 to 17 times, and in male patients with a predominance of the value in all age groups in comparison with female patients (Table 2).

Thus, the analysis of the data showed that there are differences in pathological changes in blood parameters depending on the gender of the patients. There is a high incidence of deep vein thrombosis and pulmonary embolism, and a high mortality rate in patients despite the use of standard doses of low molecular weight heparin (LMWH) recommended by the International Society for Thrombosis and Hemostasis (ISTH) and the American Society of Hematology (ASH).

At the same time, at the present stage, an adequate effective dosage has not been determined, which, in our opinion, is associated with a dead-end concept of vascular thromboembolism. The most informative are the blood levels of D-dimer protein and ferritin, which sharply differ from the normal values. In men with a fatal outcome as a result of COVID-19, this figure exceeds the norm by up to 17 times, in women from 3 to 4 times. PTT in men with a lethal outcome is within the normal range; in women it exceeds the norm by 1.5 times.

PT in women corresponds to norm, in men it is 30% lower. The number of platelets in women is below normal in 90%, in men it is higher than normal in only 1 case. Fibrinogen in men in some cases slightly exceeds the norm, and in women it is below the norm in 70% of cases. A decrease in platelet counts was observed in deceased intensive care patients compared with patients who were not treated in intensive care unit.

These data indicate that DIC is a syndrome against the background of SARS-CoV-2 infection and the development of an aggressive form of COVID-19, accompanied by the formation of structures that disrupt the patency of small and large vessels of the visceral and nervous systems may have other mechanisms and participants in pathogenetic manifestations. Some blood clotting parameters may be below normal as a result of anticoagulation treatment.

Discussion. The features of coagulopathy associated with COVID-19 have been noted in many studies [1]. Therefore, despite the fact that there is prophylactic anticoagulant therapy, there is an urgent need for studies of the real pathogenesis of thromboembolic complications and thromboprophylaxis strategies in the conservative treatment of SARS-CoV-2 infection. A noticeable increase in the level of D-dimer is associated with the development of intravascular thromboembolism (VTE) and can be used to predict and identify the risk of complications. Pulmonary microvascular thrombosis may be involved in progressive pulmonary failure. In COVID-19, the main risk factors for the development of complications leading to death are old age, male sex and the presence of concomitant diseases, especially arterial hypertension [2,10]. The lack of understanding of the pathophysiology of COVID-19, as well as the definition of an effective therapy strategy, is accompanied by severe clinical manifestations and poor outcomes in a large number of patients. The standard or increased dosage of LMWH in the group of resuscitated patients, venous and arterial thrombotic events indicate the need to revise the pathogenesis of COVID-19. Further research is urgently needed to investigate the mechanisms of SARS-CoV-2 aggression leading to thrombotic complications.

***Table 1.*** *Distribution of hospitalized patients with severe manifestations of coronavirus infection by the groups of age and gender*

| № of group* | Age | Men | Women | Total | Recovered | Died |
|---|---|---|---|---|---|---|
| | | | | | m/w | m/w |
| VII | 21–30 | 1 | 1 | 2 | 1/1 | 0/0 |
| VIII | 31–40 | 2 | 4 | 6 | 2/4 | 0/0 |
| IX | 41–50 | 9 | 4 | 13 | 5/4 | 4/0 |
| X | 51–60 | 8 | 6 | 14 | 6/3 | 3/2 |
| XI | 61–70 | 7 | 5 | 12 | 0/2 | 7/3 |
| XII | 71–80 | 6 | 5 | 11 | 0/0 | 6/5 |
| XIII | More than 80 | 1 | 3 | 4 | 1/0 | 0/3 |
| Total | | 34 | 28 | 62 | 15/14 | 19/14 |

** Official international numbering of age groups distribution*

***Table 2.*** *Indicators of blood clotting in patients with different outcomes of the disease*

| Blood count rates | Norm | | Recovered | | Died | |
|---|---|---|---|---|---|---|
| | Men | Women | Men | Women | Men | Women |
| | | | 15 | 14 | 19 | 14 |
| D-dimer, μg / μl | 0–0.5 | 0 | 0 | 0 | 1.14–8,8 | 1.47–2.44 |
| PTT, Sec. | 11 | 16 | 10.4 | 15.3 | 12.4–15.4 | 11.7–26.1 |
| PTI, % | 80 | 105 | 102 | 80–98 | 52.8–66.7 | 81.5–91 |
| Thrombocytes Units / μl | 200–400 | 180–320 | 300 | 240 | 208–478 | 133–170 |
| Fibrinogen, g/l | 2–4 | 2–4 | 2 | 3 | 2.45–4.8 | 1.29–2.51 |
| Ferritin, ng/mL | 30–250 | 10–125 | 64 | 59 | 152 | 966–1154 |

## CONCLUSIONS

1. A large number of arterial and venous thromboembolism diagnosed within 24-48 hours after hospitalization, high mortality among patients with COVID-19 suggest an urgent need to study the specific mechanisms of DIC-syndrome in SARS-CoV-2 infection and develop pathogenetically informed strategy of conservative treatment, as well as justification of the efficacy and safety of thrombi prophylaxis in outpatients with COVID-19.
2. An increase in D-dimer levels is the most informative and most significant change in blood coagulation parameters in patients with severe COVID-19, and gradually increasing values can be used as a predictor of a worse outcome.
3. The pathophysiology and mechanisms of coagulopathy in COVID-19 are not associated with the mechanisms of blood coagulation, but complicate their course.

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# A RISK-ORIENTED APPROACH TO PATIENT–DOCTOR COMMUNICATION: AN ANALYTICAL REVIEW

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**ABSTRACT** — Studying one of the most relevant issues within the context of healthcare digitization – the doctor-patient communication – offers a significant potential for finding solutions related to prevention of risks that are constantly present given the shortage of resources in healthcare. In view of the issue in question, there are ethical, legal, socio-psychological, and technological risks that are described here within the communication framework. The authors offer a view at the outcomes of a structural and functional analysis concerning these communication risks and possible options for their prevention within the context of the basic doctor-patient relationship models.

**KEYWORDS** — doctor-patient communication, communication risks, doctor-patient interaction model.

Healthcare system is a vitally important social institution that requires close attention and studying, while its dynamic progress has to face a number of issues that remain unresolved. These issues constitute risks — events or a group of related random events that cause damage to the object to which the risk pertains [1] under complex and constantly progressing social relationships. Studying of the communication between the doctor and the patient, seen as the main actors through the process of medical assistance, features a significant potential in terms of finding solutions to prevent and overcome the continuously emerging risks.

A special place here belongs to the interaction between patients and medical professionals, which makes it especially relevant to consider it through the prism of risk management, i.e. a system of measures that will mitigate the negative impact that the danger may have on the health and life of the risk taker. Potential risk is an inevitable part of people's daily routine as any action or situation of choice contains risk potential. Social changes due to research-and-technology as well as communication progress resulted in the development of a *special arrangement of public life* — a risk-based society [2]. Risks are to be observed in various types of human activity, including healthcare, where communicational interaction between the doctor and the patient is of particular importance.

A rational attitude towards studying risk issues, as well as prompt application of knowledge and practical recommendations in social healthcare, helps explain a proper behavior technology in real situations involving ethical, legal, socio-psychological and technological risks through the doctor–patient communication. Creating a fundamental risk concept involves an interdisciplinary synthesis of various theoretical models that would reflect the patterns and mechanisms of risk thinking and behavior in various fields. Accordingly, the methodology for risk processing should be based on the image of open rationality, which implies polyvariety, a shift away from the concepts of rigid determinism, which feature a strictly determined pattern of relationships and dependencies, and exclude any alternative.

*The purpose of this study* is to analyze the relevant risks within the healthcare system employing the example of communication between the patient and the doctor within the risk-based methodology context.

In this context, there can be a special interest in the health risks classification proposed by V.Z. Kucherenko and N. V. Eckert, since it allows identifying socio-legal, psychoemotional and technogenic risks as the major ones [3]. This classification sets certain vectors for working with these issues and provides the potential for further research. Following the structure of health risks, we can identify ethical, legal, socio-psychological and technological risks of the doctor–patient communication. Within the context of this study, we are going to examine the risks of the conventional (face-to-face) interaction between the doctor and the patient as well as its modern option, online.

## ETHICAL & LEGAL RISKS

An analysis of the modern healthcare system reveals this current trend: the faster the progress of the technical support offered to medical activities, the less attention is paid to the patient's personality. Certain

doctors believe that a precise examination technology eliminates the need for the doctor to communicate with the patient. Therefore, students nowadays are also taught a *light* model of medicine, where the patient is a set of impaired functional systems that are to be subjected to a certain diagnostics procedure. The society is more interested in new developments within medical technology rather than in the art of the doctor–patient communication. However, the top condition to ensure effective treatment is to establish a psychological contact and trust between the doctor and the patient. In 2003, for instance, a study was held embracing 6 countries (USA, UK, Canada, Germany, South Africa and Japan), the focus of that being doctors' (1,201 persons) and patients' (2,506 persons) view on the role of the relationships they share. One of the conclusions the study produced was the following: *The doctor–patient relationship is one of the major grounds for the stability of the society, and ranks second most important, giving way to family relations only, in all of the countries involved* [4].

The pluralism of the contemporary doctor–patient communication models as well as the lack of reflection on the types of implementing these models, the principles on which they are based, may result in a situation where values can be substituted and, in view of the modern biotechnological progress in medicine, in an expansion of the technical model. Given this situation, the requirements for a modern doctor have changed, too. The presence of illnesses, fear, psychological discomfort, lack or insufficient medical knowledge, make the patient more vulnerable and unprotected through the process of interacting with the doctor [5]. The doctor, on the contrary, is the main actor in medical practice, which means it is the doctor who is to initiate cooperation with the patient, which should be done based on trust, a peer-to-peer attitude, mutual interest, equality and active involvement of both parties.

The recent years have witnessed a significant growth in the number of claims against healthcare. The number of printed items containing negative feedback on medical topics is increasing year after year [6]. Russians now much more often contact the Investigation Committee complaining of medical issues. In 2019, over 6.5 thousand reports of improper medical care were filed, and, compared to 2018, twice as many patients came to the Committee in person to file complaints; 2.1 thousand criminal cases were initiated, with 332 cases proceeding to courts (10% more than a year earlier) [7].

The above shows that modern medicine demonstrates a transformation of the axiological communication field within the doctor–patient dyad. This trend confirms: first, the growing potential of medicine nowadays in terms of implementing its role; second, the transformation of value attitudes and patient's orientation towards more productive and competent cooperation with the doctor within the system of therapeutic relations; third, a change in attitudes and orientation of medical professionals towards technologization of patient care; declining interest from the doctor to a common human contact with the patient; reluctance to listen to the patient and express compassion and mercy.

The practice of the recent decades has featured a common situation where patients shift the entire responsibility for an unfavorable treatment outcome onto medical professionals. This is why it is important to enhance the institution of the patient's responsibility for their treatment outcomes. If an expert evaluation of the medical care quality proves that the patient failed to duly follow the doctor's recommendations, then the blame for the unsatisfactory quality of the care should reside in the patient alone [8].

It is rather common that the responsibility for negative treatment outcomes is shifted onto the doctor, there has spread a negative practice of illegal attacks on doctors undertaken by patients. This phenomenon is an effect of a weak development of legal tools that could be employed to protect doctors. It is an especially relevant issue concerning emergency care doctors. As reported by the Russian Ministry of Health, there have been over 1,200 cases of attacks on health workers registered since 2010. 200 of such cases are recorded annually in Moscow. At the same time, only 10% of the perpetrators are brought to criminal responsibility, and another 18% — to administrative responsibility [9]. The actions taken by medical employees often feature (for various reasons) violations of the provisions guaranteed by the Consumer protection law as well as by various acts regulating the procedure for offering medical care. In rare cases, medical workers themselves may die never getting due assistance to come in time [10], which is a violation of the right to medical care, subject to Article 41 of the Russian Constitution, and Article 19 of Federal Law # 323-FZ of 21/11/2011 "On the basics of public health protection in the Russian Federation" [11]. It is to be noted that there is an increasing aggression coming from patients who do not trust medical specialists and, moreover, deliberately neglect their recommendations, which undermines the authority of the medical community in the eyes of the public [12].

In this situation, health care workers do not make sufficient use of the available legal mechanisms to protect themselves from such attacks. In light of this, it seems a reasonable idea to empower medical professionals enabling them to prevent such actions. How-

ever, in 2016, Tatyana Yakovleva (Deputy Head of the Russian Ministry of Health) emphasized that it is *only a phonendoscope that a doctor should have in the hands* [13]. Despite the amendments to the Criminal and Administrative Codes, meant to offer legal protection of healthcare employees [14], which imply penalties for counteracting to lawful actions taken by a healthcare professional offering medical assistance (at the time this article was being written, there was still no legally established court practice aimed to follow the provisions of Article 6.3.6 of the Administrative Code and Article 124.1 of the Criminal Code), so doctors remain basically unprotected as they enjoy no right to respond immediately whenever there is a threat to their lives and health.

This reality urges a need for public and professional discussion on potential use of non-legal solutions when medical professionals stay performing their medical duties. Besides, the respective management should assist in filing claims against cases of insulting doctors' honor and dignity, as well as in cases where doctors' rights were violated. The protection of honor and dignity, professional reputation is done on a common basis, which is obvious from the practice adopted by the Supreme Court of the Russian Federation [15]. These aspects make the doctor and the patient unequal members within the respective legal interaction, since the doctor is deprived of certain tools to work an impact on the patient and to control the treatment and diagnostics process, namely: employing the medical community's authority to encourage the patient to listen to the doctor's opinion; deliberate non-disclosure of certain information to the patient concerning unsatisfactory treatment outcomes or full disclosure of the risks and effects of the upcoming treatment; using differences in the way various social groups perceive medical information, where, due to numerous factors (low standard of living; information from the media that discrediting medical employees; previous negative experience) shape a negative attitude among patients. This affects not doctors alone, yet also patients, who, in turn, are deprived of competent and timely medical assistance and empathy from medical staff, which is due to the progressive introduction of the *technogenic approach* in healthcare.

Another factor is the risk of trust in medical staff, which creates socio-psychological basis for the emergence of pseudo-medical organizations that are engaged in commercial activities exclusively, yet claim to be involved in medical activities. An example of that is an incident in the Khabarovsk Region of Russia, when offenders rented area in large shopping malls to install pseudo-medical equipment there. Specially hired call-center employees would phone common people inviting them to a comprehensive diagnostic procedure, which was offered on a free basis. The impostors had no medical degree, yet they set some grave diagnoses further offering people treatment in their clinic [16]. This demonstrates that the emergence and expanding influence of such organizations will result in a declining authority of medical professionals, poor trust towards the medical community, and will entail the risk of further growth of distrust in the social Institute of medicine as a whole.

Along with the problems mentioned above, there is also note to be made regarding the growing commercialization of healthcare. Nowadays, primary care doctors and pharmaceutical companies/pharmacies enter some unwritten agreement on mutually beneficial cooperation, following which doctors (in many cases this happens in the primary healthcare system) will recommend or even insist openly that the patient buy a particular drug, for which the doctor will enjoy some bonus [17]. The lawmakers in this case followed the principle of prohibition, which means that anything that is not banned directly by law is allowed, thus opening up a wide path for cooperation, as well as for the development of medicine and pharmaceutical industry [18]. At the same time, according to the current legislation, there are restrictions in place concerning the following: representatives of pharmaceutical companies visiting medical staff in violation of the local order approved at the medical institution; transfer of promotional samples to medical employees; direct transfer of funds to medical professionals (which can be confirmed through e-correspondence) with no contract signed, which would imply carrying out clinical research or academic/scientific activities. The list of restrictions is quite large and the legislation implies liability for violating the above-mentioned provisions [19].

In view of the above, mutual violation of rights by both the doctor and the patient will make it complicated for medical employees to perform within their professional duties, as well as it will also increase the potential for risks, regardless of the interaction model within the doctor–patient dyad.

## SOCIAL AND PSYCHOLOGICAL RISKS

While examining this category of risks within the doctor–patient interaction context, attention should be paid to the doctor–patient communication issues, which manifest themselves through the doctor's not understanding the patient. On the patient's part, it is about lack of willingness to listen, and, most importantly, to hear the doctor and to follow their recommendation [20], which then lays ground for a conflict between the expectation and the reality, and which is one of the reasons behind conflict situations.

Given the time limit on an appointment by the doctor (regulated by Order of Ministry of Health of Russia, 02/06/2015 # 290n "On approval of the standard industry time limits for the performance of activities related to one patient's visit to the local Pediatrician, General Practitioner (family doctor), Neurologist, Otolaryngologist, Ophthalmologist, and Obstetrician-Gynecologist", doctors fail to offer their patients as much attention and time as they would like to.

It is reasonable for medical professionals to master the skills of medical rhetoric, since this is a highly effective communication tool. The key element of rhetoric is how the doctor communicates with the patient. The doctor then has the task of correctly balancing between confidential medical data and the information that can be disclosed to the patient. When interacting with a patient, the doctor in most cases will use special terms and choose the right behavior tactics, while bearing in mind that it can cause not only a positive response, yet also provoke a psychological trauma [21].

## TECHNOLOGICAL RISKS

Out of a significant number of technological issues within this study, there is a focus to be made on designing effective online communication between the patient and medical specialists. From this stance, general technological risks, both on the doctor's and on the patient's part, will be considered. A set of means and tools for online communication is now called telemedicine. Subject to Par. 22 Art. 2 of Federal Law # 323-FZ of 21/11/2011 "On the Basics of Public Health Protection in the Russian Federation", telemedicine technologies include information technologies that provide remote interaction of medical workers with each other, with patients and (or) their legal representatives, identification and authentication of these persons, documentation of their actions through consultations, meetings, remote medical monitoring of the patient's health.

The major areas to apply the regulation act are electronic prescriptions for medicines containing narcotic and psychotropic substances; obtaining voluntary consent to medical care following a simplified pattern; remote execution of the patient's right to obtain medical data regarding themselves; updating medical care standards in view of advanced technologies; legislative validation of remote consultations.

There is basically no data available on the results of research carried out in the field of telemedicine technologies and published in peer-reviewed journals. A number of authors believe that Russia's medical science has considerable experience in developments within the area of telemetric medicine [22]. For medical employees such risks include: professional training, retraining of doctors to be further employed in the sphere of online communication system; legal and insurance protection for doctors involved in telemedicine; clinical practice.

As for patients, these risks include: protection for patient databases and the overall lack of security for personal data and information that constitutes medical and any other professional secrecy. This issue may be solved through blockchain technologies enabling to confirm transactions of a counseling offered by a medical specialist, as well as to issue medical documents [26]. To date imperfect telemedicine and diagnostic technologies presents a risk in diagnosis and delivering diagnostic data to the doctor with inaccuracies and critical errors [23].

Nowadays, the Internet of medical things has been developing actively; this is a network that combines devices into a computer network and allows them to collect, analyze, process and transmit data to other objects through software, applications or technical devices [24]. This area is a concept of a network that joins together *connected devices* and devices that monitor the status of the human body and its environment, including medical devices that can have an interactive influence on the prevention, treatment and rehabilitation.

One of the options to achieve this goal is the concept of *Geographical information system (GIS) of a human being*, which offers a whole new approach to collecting most comprehensive data concerning a person, which would offer the basis for drawing conclusions regarding the body status. GIS collects information about the person's status as well as about their environment (both from an ecological point of view and from a social one) [25]. The Internet of medical things will take explanation with the clinical efficacy and safety evaluated, which would require clinical studies. Rapid and large-scale introduction of advanced technologies takes training medical professionals and patients to develop respective user skills [26].

The context of the doctor–patient interaction involves three groups of issues: ethical and legal, socio-psychological, and technological. Now, further comprehension of the issues under consideration, will take analyzing the existing interaction models within the patient-doctor dyad, as well as examining a risk analysis as per each model. First of all, it is important to have a quick overview of the already available doctor–patient interaction models.

Now, it is to be seen that the main model is the contract system and its evolutionary development — equal responsibility for the treatment outcome shared by the doctor and the patient. Each of these issues

involves risks that should be taken into account when arranging and offering medical care, as well as when planning programs for the development of the health sector (Table 1).

— digitization of healthcare has not just changed the conventional configuration of the doctor–patient communication, yet also brought around its

***Table 1.*** *Communication risk analysis and possible options to prevent them within various doctor-patient interaction models*

| Model | Features | Communication risks and possible ways to prevent them | | |
|---|---|---|---|---|
| | | Ethical & legal | Social & psychological | Technological |
| Technical | The patient is a mechanism that has broken down. The patient's personality and individual features are not taken into account. | The doctor is responsible for the negative outcomes that occurred due to their fault, which creates the risk of reputational loss.<br>Potential use of technologies without prior consent from the patient.<br>Risk prevention is ensured through a documented consent from the patient agreeing to accept medical assistance, and the doctor's liability disclaimer in case of force majeure.<br>There is a need to convey to patients as much information as possible in a respectively comprehensible language. | Dissatisfaction with the treatment quality due to lack of emotional contact with the doctor.<br>Risk prevention is based on informing the patient as much as possible regarding the content of the medical assistance and its effects for a particular patient. | Reduced potential for effective feedback while monitoring long-term effects of medical care.<br><br>Risk prevention is based on interactive online communication services introduced in medical practice. |
| Paternalist (sacral) | The doctor is the parent, and the patient is an unreasonable child | The patient bears no responsibility for their own health. It is the doctor who is fully responsible for the patient's health.<br>Possible use of technologies without obtaining the patient's consent.<br>The basic foundation for both the doctor and the patient is ethical regulation (deontology). | Risk, either intentional or not, of the doctor's working harm to the patient.<br>Compliance with ethical (deontological) principles guarantees the patient's safety.<br>Risk prevention is associated with reaching psychological comfort for the patient and depends on the traditions of medical care, as well as on the role and authority that the doctor enjoys in the society. | The patient has little, if any, access to the examination outcomes.<br>Possible use of technologies with unproven efficiency.<br>Risk prevention is based on the introduction into medical practice of methods with proven effectiveness and technologies for interactive communication with patients. |
| Collegial | This model is patient-centered. The patient's role is active, and all the decisions that the doctor makes are to be discussed with the patient. | The patient and the doctor share equally the responsibility both for the course and results of treatment.<br>The major risk within this model is lack of a properly designed contract for medical services.<br>Ethical risks are minimal, yet depend on the degree of the contact between the doctor and the patient.<br>Risk prevention is based on reaching a balance between the interests of the patient and those of the doctor, subject to a contract. | As of today, this is the most attractive model from a psychological point of view. However, there is a risk of the patient's independent selecting the treatment strategy never taking the doctor's advice.<br>Professional support for the doctor's decisions offered by the medical community would be reasonable as part of risk prevention, especially in complex and conflict situations. | The patient may reject the use the necessary technologies.<br>Possible neglect of the doctor's recommendations and deliberate disconnection of health monitoring devices.<br>Potential disclosure of personal data when used through remote counseling and data exchange;<br>Risk prevention is associated with interactive technologies introduced to ensure communication between patients and medical experts. |

## CONCLUSION

Summarizing the above can be boiled down to the following key points:

new format, while modifying the axiological field of the interaction occurring between the doctor and the patient. First, there is an intermediary between

the doctor and the patient – the medical content of the Internet. Second, online services for patients are developing. The progress of digital healthcare is transforming modern medicine, which will take change in the way the roles of doctors and patients are perceived. New risk factors are associated with the patient's active involvement in the treatment process, the constant availability of social network support, rapid data transmission and an open two-way dialogue. Blogs, social networks, online counseling, etc., all these generate new interaction models between doctors and patients, medical organizations and other medical subjects, which creates a new interaction environment that has yet to be studied. They will not, of course, replace the traditional doctor–patient interaction. The potential they offer, though, acting as platforms for shaping personalized medicine (offering patients respective information, access obtaining counseling with another specialist), contribute to making better decisions concerning health;

— the doctor–patient communication viewed within the digital healthcare system features the following advantages: instant feedback, access to the most up-to-date medical information in a real time mode, stable access through social networks, transparency of information for the patient, its availability at a distance, two-way dialogue in real time. However, while there are advantages, the doctor–patient interaction in the e-health system gives rise to additional risks related to personal data protection, as well as to the quality and reliability of medical information. Therefore, communication between a doctor and a patient, seen as a special type of social activity, includes a risk component, which can not only harm the major actors involved in the process of providing medical care, yet can shape a whole set of healthcare practice issues of varying complexity;

— the contract system and its evolutionary development is currently considered to be the basic model of doctor–patient interaction. This is a model where the doctor and the patient share the equal responsibility of for the treatment outcome. Ethical, legal and socio-psychological risks are reduced to the minimum through employing an agreement as the major document regulating the communication between the doctor and the patient. At the same time, the humanistic component of medical activity remains intact — technicism as an ethical and legal risk is actually leveled by the provisions of the agreement. The main risk in this case is the refusal to enter or follow the agreement, especially in cases requiring emergency medical assistance. Besides, there is also a significant risk of the patient's refusal to employ advanced technologies when it may be necessary;

— nowadays, there are a number of issues involving the safety of health workers, namely, ambulance medical workers. Given this context, it appears urgent to have a parliamentary discussion concerning allowing medical employees using special means of protection, as well as regulating the procedure to use them;

— online communication technologies have gained legislative support and are actively progressing now. However, implementing them faces a number of serious issues related to the protection of personal and other legally protected data; socio-psychological features of the doctor–patient interaction when using them.

The analysis of doctor–patient interaction models has allowed us to identify three major communication risk groups (ethical & legal, social & psychological, technological) and to propose some solutions. We assume that this approach should be used to make healthcare more professional, efficient and personalized.

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# PERSONALIZED CALCULATOR FOR PREDICTION OF OPIOID-ASSOCIATED PHARMACORESISTANCE IN PATIENTS WITH PANCREASE CANCER

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**ABSTRACT** — We studied the complex effect of genetic and non-genetic factors on the formation of opioid-associated resistance using machine learning methods in patients with chronic pain syndrome against the background of pancreatic cancer. Fifty-seven factors for predicting the realization of pharmacoresistance were studied in all examined Caucasian patients. The most significant predictive factors were determined and a software-analytical complex "Algorithm for assessing the significance of clinical and pathogenetic factors for predicting the safety of opioid therapy" was developed.

**KEYWORDS** — chronic pain syndrome; pancreas cancer; pharmacoresistance, fentanyl TTS; forecasting.

## INTRODUCTION

Opioid-associated adverse reactions (HP) are recorded in 49–95% of cases in cancer patients [1]. Pancreas cancer predetermines the obligatory use of strong opioid analgesics in palliative care [2]. A feature of the pain syndrome of pancreatogenic genesis is the development of resistance of opioid analgesics against the background of progression of the underlying disease. In the literature, there are isolated conflicting studies on the study of the complex effect of genetic and nongenetic factors on the implementation of the resistance of opioid analgesics. A personalized comprehensive assessment of predictor factors for the realization of opioid-associated resistance in patients with pancreas cancer will increase the effectiveness and safety of analgesic therapy.

*Purpose of the study:*

To develop a calculator for personalized risk assessment of opioid-associated drug resistance in patients with pancreas cancer using fentanyl TTS as an example.

## MATERIALS AND METHODS

90 patients (Caucasians, residents of the Krasnoyarsk Territory of Eastern Siberia) with pancreas cancer on the background of existing chronic pain syndrome (male to female ratio 1: 1) at the age of 18–75 were examined. The median age of the examined patients was 63 (56–69) years. The intensity of chronic pain syndrome according to the digital rating scale was 6 (6–8) points at the time of inclusion in the study. In 80% of the cases, mixed pain syndrome prevailed, in 20% — no ciceptiveone, respectively. All the patients received fentanyl TTS for pain relief against the background of standard analgesic therapy (ketoprofen 300 mg / day, diazepam 10 mg / day, amitriptyline 25 mg / day).13 geneticfactors(*ABCB1* (rs1045642, rs2032582, rs1128503); *OPRM1* (rs 1799971); *UGT2B7* (rs 7668258, rs12233719, rs7438135); *CYP3A4*1B* (rs2740574); *CYP3A5*3* (rs776746); *CYP3A4*22* (rs35599367); *IL1B* (rs1143627); *PTGS2* (rs5275); *LOC541472* (rs1800795)), 20 clinical and demographicones (gender; age; localization of pancreas cancer; pathogenetic variant of chronic pain 0/6 (0 — start of treatment, 6 — six months of treatment); type of surgical treatment (radical / palliative; physical status on the ECOG scale 0/6; jaundice; cancer-associated weakness syndrome; dyspepsia; comorbidity; ascites; body mass index 0/6; mental status on the MMSE 0/6 scale; life quality indicators on the ESAS 0/6 scale; stage of pancreas cancer, and 24 laboratoryones(glomerular filtration rate 0/6, aspartate aminotransferase 0/6, alanine aminotransferase 0/6, bilirubin 0/6, total protein 0/6, hemoglobin 0/6, leukocytes 0/6, lymphocytes 0/6, platelets 0/6, erythrocytes 0/6, glucose 0/6), amylase 0/6)were analyzed as the studied predictive factors. As part of the accompanying treatment, indices and opioid metabolism inhibitors were excluded as much as possible to reduce the risk of drug interactions. The assessment of the reliability of the development of pharmacoresistance was carried out according to the Naranjo scale and using the algorithms of Karch F.E.,

Lasagna L. [3]. Quality of life was assessed using the Palliative Medicine Symptom Rating Scale (ESAS), and cognitive functions were assessed using the Mental Status Assessment Scale (MMSE). The observation period was 5.95 ± 0.67 months. The following machine methods using the Scikit-learn library (Python) were used as predictive models to determine the likelihood of drug resistance: logistic regression (LR); k — nearest neighbors algorithm for classification problem (KNC); a collective of decision trees by the "random forest" method for the classification problem (RFC); a collective of decision trees by the gradient boosting method for the classification problem (GBC); decision trees for the classification problem (DTC); artificial neural network (multilayer perceptron) for classification problem (MLPC); linear support vector machine for classification problem (LSVC); support vector machine for classification problem (SVC). As a result of machine learning methods using 50 runs in each predictive model, the best quality models were selected using the least number of predictive factors. The model was trained on the entire training data set. The model was checked for quality on a test dataset. The most effective model was taken to be a model that achieves high accuracy with a minimum set of features. Statistical processing of the research results was carried out using the IBM SPSS® Statistics 20.0 software (USA). Differences were considered significant at a significance level of $p < 0.05$.

## RESULTS

The most effective model was the support vector machine for the classification problem. For predicting pharmacoresistance, this model used only 4 predictor factors out of 12 possible and was highly reliable ($p = 0.000$). SVC has demonstrated the technological and practical advantage of the algorithms used. The list of important predictors of the implementation of pharmacoresistance includes genetic and non-genetic factors with a certain rank significance (Fig. 1)

The final stage of the study was the development of a software-analytical complex "Algorithm for assessing the significance of clinical and pathogenetic factors for predicting the safety of opioid therapy" in order to support decision-making to ensure the safety of opioid therapy. As a result, the mutual influence of only four predictor factors determined the risk of fentanyl-associated pharmacoresistance realization. (Fig. 2).

## DISCUSSION

The frequency indicators of pharmacoresistance of 17 people (18.89%) in this study predetermined the personalized modeling of its implementation based on clinical and genetic factors. The developed model for a comprehensive assessment of the factors of the implementation of pharmacoresistance will allow monitoring the effectiveness and safety of opioid therapy, and will also ensure the availability and timeliness of the use of interventional methods of analgesia when therapy is ineffective. Genetic factors have become the leading predictors in the created integrated model for predicting pharmacoresistance to opioid therapy using fentanyl TTS as an example. Homo- and heterozygotic carriage of one-nucleotide variants (ONV) of the *PTGS2* gene (AA and AG rs5275) and the *IL1B* gene (AA rs1143627) can provide the development of pharmacoresistance due to the implementation of a multicomponent inflammatory mechanism of chronic pain in cancer patients. ONV gene *ABCB1* (GG RS1045642) showed a significant role in progosingpharmacore resistance. ONV gene *ABCB1* (GG rs1045642) showed a significant role in progosing-pharmacoresistance. It is necessary to consider the possibility of extracellular acidosis of the performance space on increasing the functional activity of P-glycoprotein due to hypoxia [4]. The lack of predictive value for laboratory parameters based on the results of machine learning and testing can be explained by the features of the safe pharmacokinetics of the transdermal therapeutic system in comparison with other non-invasive forms of opioids and the mutual influence of the obtained predictors.

## CONCLUSION

The results of the study predetermine the obligatory pharmacogenetic study for patients with pancreas cancer. The use of risk stratification in the software-analytical complex for the development of pharmacoresistance predetermines the improvement of the personalized approach to pain relief in patients with pancreatic cancer.

*Contributors*

Bobrova Olga designed the study and analysed and interpreted data. Sergey Zyryanov and Marina Petrova interpreted and analysed the data. Natalya Schneider, Bobrova Olga, Sergey Zyryanov, Marina Petrova prepared the manuscript for submission.

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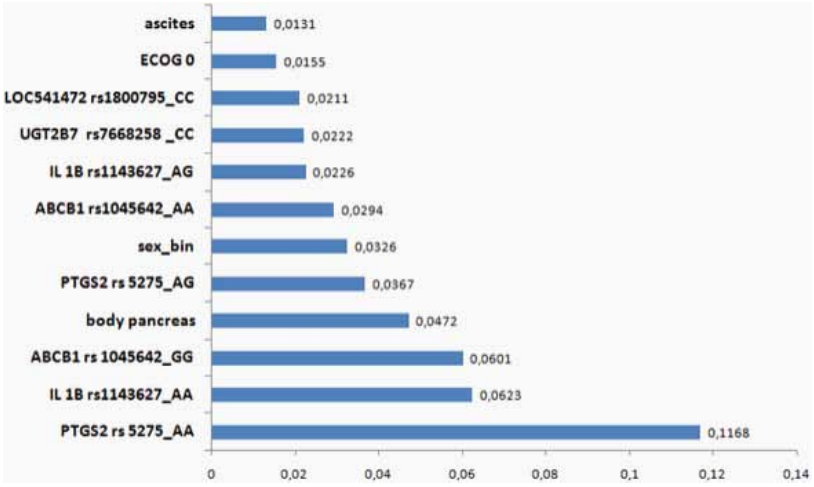


***Fig. 1.*** *The significance of the selected SVC traits for predicting pharmacoresistance in patients with pancreatic cancer during therapy with fentanyl TTS.*
***Abbreviations:*** *TTS — transdermal therapeutic system; ECOG — scale for determining physical status; SVC — support vector machine predictive model for classification problem.*

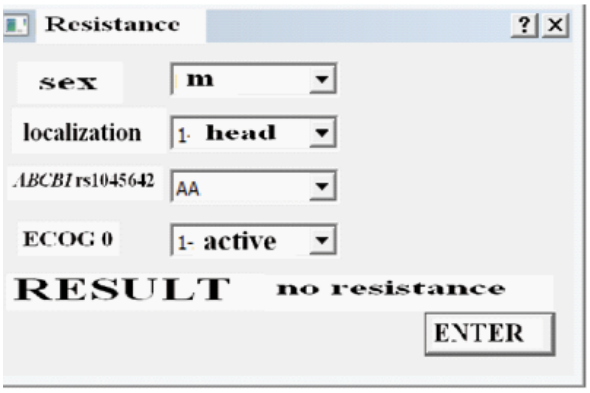


***Fig. 2.*** *Clinical and genetic risk meter for the implementation of fentanyl-associated opioid resistance in patients with pancreatic cancer*

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# MARKERS OF ENDOGENOUS INTOXICATION AND OXIDATIVE STRESS IN PATIENTS WITH OPIOID DEPENDENCE SYNDROME

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**ABSTRACT** — Aim of study is to evaluate changes in fluorescence indicators that reflect the endotoxicosis and oxidative stress status in patients with opioid dependence syndrome through treatment.
Materials and methods. The study involved 28 patients with opioid dependence syndrome and 20 healthy persons. Fluorescence parameters reflecting the accumulation of proteins oxidative modification products were determined in the blood plasma.
Results and discussion. At the stage of admitting the patients to the hospital, the blood plasma was found to contain increased levels of FLOPs and bityrosine residues by 18–30% against 12–20% reduction of the intensity of intrinsic and probe fluorescence of proteins. At the same time, upon the completion of the therapy course (15–17 days) all the studied parameters returned to the values those in the control group, except for the level of bityrosine residues, which remained 20% above that in the control. This may be accounted for by the process of updating plasma proteins and the greater stability demonstrated by bityrosine molecules.
Conclusion. FLOPs and tyrosine residue identification can be used for laboratory monitoring of early response to the therapy as well as for a long-term efficiency follow-up in patients with opioid dependence syndrome.

**KEYWORDS** — dependence syndrome, opioids, drug addiction, laboratory diagnostics, oxidative stress, blood plasma fluorescence.

## INTRODUCTION

To date, a growing dependence on psychoactive substances presents an unfavorable trend of an increase in related morbidity and mortality. According to the United Nations office on drugs and crime, up to 272 million people have tried illicit substances at least once in 2010, and about 200,000 people die annualy from drug-related issues [1]. One of the most promising laboratory markers in addiction-treatment practice are the indicators of the intensity of the inflammatory response, as well as indicators of vascular damage, which also include oxidative stress markers [2, 3]. The link between chronic opioid exposure and oxidative stress has been shown in experimental studies, which in particular focus on the development of mitochondrial dysfunction, an increased production of the reactive oxygen species in the mouse brain mitochondria, lipid peroxidation products, and the presence of carbonyl protein residues along with a decrease in the glutathione concentration [4]. The data from clinical studies also indicate on a significant role that oxidative stress plays in etiology of the dependence syndrome. At the same time, we have not found enough explanation on the use of markers of free radical activity and the functional status of the antioxidant defense system in laboratory monitoring and prediction of drug pathology [5, 6].

*Aim of study*

to evaluate changes in fluorescence indicators that reflect the endotoxicosis and oxidative stress status in patients with opioid dependence syndrome during the treatment.

## MATERIALS AND METHODS

The study was carried out using biological material (peripheral blood) from patients with opioid dependence syndrome at the Krasnodar Region Addiction Clinic. There were a total of 28 patients and a control group comprised 20 relatively healthy individuals. Blood was collected from the patients by the first day after admission and prior to their discharge. The average treatment length was 15–17 days.

The treatment was aimed at correcting the major mental disorders. In blood plasma, we measured the intensity of intrinsic fluorescence of tryptophanyl proteins (excited by light at 280 nm and registering light emission at 330 nm), as well as the intensity of fluorescence (excited by light at 380 nm and registering light emission at 490 nm) of the 1,8-ANS (1-anilin-8-naphthalenesulfonic acid) probe when binding to plasma proteins, mainly albumin [7]. To assess oxidative damage, we determined the level of fluorescent products of oxidative damage of proteins (FLOPs), bityrosine,

as well as identified the fluorescent parameters typical of plasma proteins non-enzymatic glycation products (AGE). FLOPs measurements were done at the fluorescence excitation/emission wavelengths of 320/420, 360/420, and 400/475, while the parameters were marked by the excitation wavelengths (FLOP 320, FLOP 360, and FLOP 400) [8]. The content of bityrosine residue was also evaluated employing the fluorimetric method with the registration at the emission wavelength of 410 nm while excited by light at 310 nm [9]. The protein glycation products were identified under conditions of excitation by light with a wavelength of 370 nm, while recording the emission of light at a wavelength of 404 nm [10,11]. The laboratory part of the study was performed with the SM2203 spectrofluorometer (Solar, Belarus). All the measurements were carried out in a temperature-controlled cuvette at 25° C. The study was approved by the independent ethics Committee at Kuban State Medical University (Protocol #58 of 11/12/2017).

The statistical data processing was performed with AnalystSoft Inc., StatPlus statistical analysis software, Version 7 (see www.analystsoft.com/ru/). To compare the values obtained from the control group and the comparison group, the nonparametric Mann-Whitney test was employed, as well as the nonparametric Wilcoxon test, which was used to compare the values for the patients from the experimental group prior to, and after, the treatment. The differences were considered statistically significant at $p<0.05$.

## RESULTS AND DISCUSSION

The study has revealed that the patients with opioid dependence syndrome had a statistically significant growing content of products of oxidative damage of proteins which was detected through fluorescent methods. At the point of admission to the addiction clinic, the FLOPs levels exceeded the values of the respective indicators in the control group by an average of 18–30%. The blood plasma content of FLOP 320 in patients with opioid dependence syndrome exceeded the control values of the same factor by 24%, whereas the level of FLOP 360 went up by 18%, and the FLOP 400 level — by 30%.

An evaluation of the bityrosine residues content at admission also was observed at a 20% increase in the level of this oxidative protein damage product in the blood plasma. Measuring the level of accumulation of protein glycation endproducts which is a common method of controlling hyperglycemia in patients or when modeling diabetes mellitus revealed a small yet statistically significant increase in this value by 8%. This observation, however, offers an explanation to separate identification of oxidative modifications and glycation products by fluorescent methods, despite the seemingly low specifics of these methods. This result was not unexpected due to the well-known fact of the oxidative stress status in patients with drug dependence; the determination of proteins non-enzyme glycation fluorescent products seemed plausible, too, in view of this process acceleration not against hyperglycemia only, yet also in view of its strengthening during oxidation and other protein damage.

The identification of the intensity in the plasma proteins intrinsic and probe fluorescence was aimed at detecting their conformation disturbance, which can develop due to the same oxidation modifications or due to endotoxicosis, since protein molecules interact actively with other molecules, including inorganic ions, low-molecular organic compounds and peptides. The outcomes of studying the intrinsic fluorescence of tryptophan residues and the 1,8-ANS probe fluorescence in the blood plasma in patients with opioid dependence syndrome featured initially reduced values in their intensity — by 12% and 20%, respectively.

The values, obtained after therapy and analyzed through this study revealed a partial recovery, which implied an increase in the level of tryptophanyl intrinsic fluorescence and the intensity of 1.8-ANS fluorescence to the control values of the corresponding indicators. The level of FLOPs also increased at the stage the patients were discharged from the clinic, and reached normal values. Given that, the content of tyrosine residues remained initially increased by 20% after 15–17 days of therapy as well. Such results may be due to a fairly long observation period — over 2 weeks; this period is enough to ensure almost complete renewal of serum albumin molecules (average life span — 2–3 weeks) — the major protein of blood plasma, which determines the native features of the biofluid proteins fluorescence. At the same time, the bityrosine molecule is quite stable and persists through the said period.

## CONCLUSION

The study outcomes demonstrate the potential of evaluating fairly simple blood plasma fluorescent parameters enabling to examine the severity of oxidative damage to proteins. Parameters like FLOPs and bityrosine residue levels can be used for laboratory monitoring of metabolic disorders in patients with opioid dependence syndrome, as well as for monitoring the effectiveness of therapy. Given the different types of changes in the proposed markers, they may prove useful both as a short term mode — for assessing early response to therapy, and as a long-term one — to assess therapy or rehabilitation compliance.

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# THE EFFECT OF PIR-12 COMPOUND ON SURVIVAL AND NEUROLOGICAL DEFICITS IN EXPERIMENTAL GLOBAL CEREBRAL ISCHEMIA IN RATS

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**ABSTRACT** — This study was aimed to assess the effect of a new pyrimidine derivative (PIR-12 50 mg/kg) on survival and neurological deficits in rat global brain ischemia. It has been confirmed that the investigated compound PIR-12 contributes to an increase in survival up to 80% and a decrease in neurological status by 73,3% compared to the control group of animals and exceeds the strength of the effect of the reference drug Cavinton by 30% and 22,48%, respectively.

**KEYWORDS** — brain ischemia, the survival rate, neurological deficit, derivatives of pyrimidine.

## INTRODUCTION

Currently, brain ischemia has remained one of the most common pathologies in modern society [1]. With cerebrovascular brain damage, first of all, neurological disorders occur, and therefore effective and timely therapy is a key link in the treatment of this pathology. Pyrimidine derivatives have been found to possess pronounced anti-inflammatory [2], anti-aggregation [3], and endothelioprotective [4] properties, therefore, other types of pharmacological activity, including cerebroprotective activity, can be assumed for this group of substances.

*Objective:*

To study the effect of PIR-12 compound on survival and neurological deficits in experimental global cerebral ischemia in rats.

## MATERIALS AND METHODS

The study was conducted in accordance with the "Guidelines for Preclinical Trials of Drug Products" ed. by A.N. Mironov (a 2012 edition.) [5]. The animals were maintained in compliance with current best practices and standards of care in laboratory animals. The experiment was performed on 40 male Wistar rats m=220–240 g, divided into 4 groups (n=10). Rats were kept on a standard vivarium diet, with a natural succession of light and darkness. The first group was represented by falsely operated rats (FO), the second one — by negative control animals (NC). The both groups received an intraperitoneal suspension of Tween-80 in purified water. The third and fourth groups received reference drugs: Cavinton (3,2 mg/kg) [6]. The fourth group was administered the pyrimidine derivative PIR-12 (50 mg/kg) [7]. The second and subsequent groups were simulated global brain ischemia by bilateral occlusion of the common carotid arteries (under chloral hydrate anesthesia 350 mg/kg) [8, 9]. All objects were injected intraperitoneally for three days before the operation. A day after modeling the pathology, the survival rate of animals and their neurological deficit were evaluated on the McGraw scale. All findings were processed by means of variation statistics methods using the STATISTICA 6.0 software. The normality of distribution was assessed by the Shapiro-Wilk test. In the case of a normal distribution of the data, a parametric t-test was applied. In the case of abnormal distribution of the data, the statistical processing was performed using the Mann-Whitney U-test. The difference was considered significant at the significance level of more than 95% ($p<0,05$).

## RESULTS

Irreversible occlusion of the common carotid arteries led to the death of 70% of the negative control group (NC) rats, while no mortality was observed in the falsely operated animals (Fig. 1). In the surviving individuals of the NC group who did not receive pharmacological support, the degree of neurological disorders reached 7,5±0,29 points (Fig. 2), which was manifested in lethargy, slowness and Manege of movements, one- and two-sided ptosis of the eyelids, paresis of the limbs, and in some cases paralysis. Against the background of prophylactic administration of Cavinton, the mortality rate of animals reached 40%, and the neurological deficit on the McGraw scale was 2,58±0,27 points, which is 65,6% less than the same

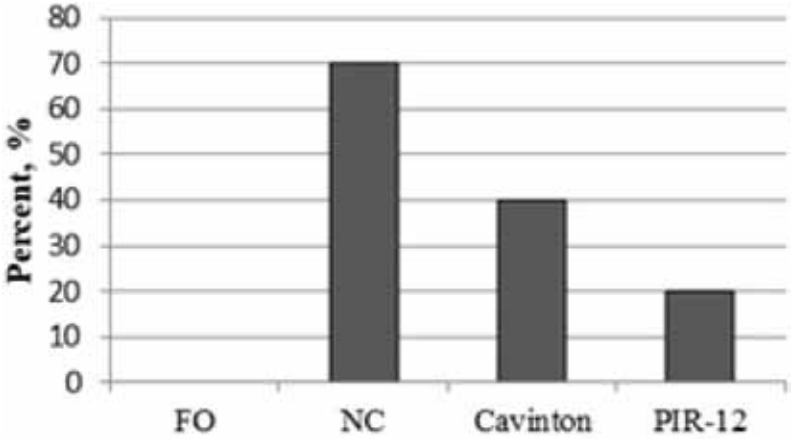


***Fig. 1.*** *Assessment of the effect of PIR-12 and Cavinton compounds on the mortality rate of global cerebral ischemia in rats.*
***Note:*** *FO — false-operated rats ; NC — negative control rats; Cavinton — a group rats treated with Cavinton; PIR-12 — a group of rats treated with PIR-12.*

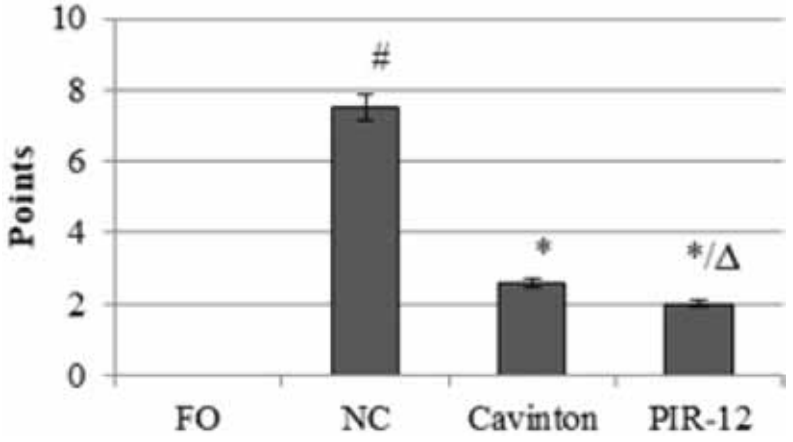


***Fig. 2.*** *Assessment of the effect of PIR-12 and Cavinton on the severity of neurological deficits in the conditions of global cerebral ischemia in rats.*
***Note:*** *FO — false-operated rats ; NC — negative control rats; Cavinton — a group of rats treated with Cavinton; PIR-12 — a group of rats treated with PIR-12; # — statistically significant as compared to the FO rats (p<0,05); * — statistically significant as compared to the NC rats (p<0,05); Δ — statistically significant as compared to rats treated with Cavinton (p<0,05).*

indicator of the NC group of rats (p<0,05). Under conditions of cerebral ischemia, the mortality rate of rats treated with intraperitoneal PIR-12 compound was 20%, while the indicators of neurological deficit decreased as much as possible relative to untreated rats by 73,3% (p<0,05). At the same time, the neurological status of PIR-12 animals was 22,48% lower than that of Cavinton rats (p<0,05), which was statistically significant.

## CONCLUSION

In the experimentally simulated cerebrovascular insufficiency, a pyrimidine derivative under the laboratory code PIR-12 allowed to compensate for survival and reduce neurological deficit, and showed an effect which exceeded in its strength that of the non-targeted drug Cavinton.

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# ANTIHYPOXIC ACTIVITY OF THE VMA-10-18 DERIVATE UNDER HYPOBARIC HYPOXIA IN MICE

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**ABSTRACT** — The present study was carried out to evaluate the effect of a new derivative VMA-10-18 (10 mg/kg) on the resistance of mice to acute hypobaric hypoxia. It was confirmed that the studied derivative contributes to an increase in the life time on the lethal test site by 2,7 times ($p < 0,05$) compared with the control group of animals and exceeds the strength of the effect of the reference drug Metaprot by 1,2 ($p < 0,05$).

**KEYWORDS** — hypobaric hypoxia, mice, derivative VMA-10-18, Metaprot.

## INTRODUCTION

The pathogenetic basis of most diseases and various kinds of conditions is hypoxia [1]. This problem plays an important role in various fields of human activities: military, sports, space, etc. [2, 3]. In people who are often exposed to hypoxia, disturbances in ion channel conductance, acid–base homeostasis are observed. This affects all systems of the body and inhibits its recovery after induced physical and mental stress.

Antihypoxic agents are used in clinical practice to support the viability of the organism in condition of hypoxia. However, the arsenal of such drugs is limited, or the breadth and effectiveness of therapeutic doses are small [2, 3, 4]. Therefore, the search for new compounds with a wider range of antihypoxic doses and the absence of side effects is an urgent task of experimental and clinical pharmacology, which served as the basis for this experiment [5, 6, 7, 8].

*Objective:*

To study the antihypoxic activity of a new derivative VMA-10-18 under conditions of hypobaric hypoxia in mice.

## MATERIALS AND METHODS

The experiment was performed in accordance with the Guidelines for preclinical studies of drugs, ed. A.N. Mironov (2012 Ed.) [9]. The animals were kept in the vivarium of the Pyatigorsk Branch of the Volgograd state medical University (Russia). The study was conducted on 30 mongrel white mice (m=20–24 g). The animals were divided into 3 groups (n=10). During the experiment, all experimental animals were kept under standard vivarium conditions (natural light change mode, temperature, relative humidity, standard diet of laboratory animals, weekly change of bedding and cages, fixed feeding and drinking times) in compliance with the International recommendations of the European Convention for the protection of vertebrates used in experimental studies. The first group — control, received a solution of sodium chloride in an equimolar volume, the second group was injected with VMA-10-18 (10 mg/kg), synthesized at the department of organic chemistry of the Pyatigorsk Medical and Pharmaceutical Institute (Russia) ; the third — the reference drug Metaprot (JSC «Pharmproject», Russia) (25 mg/kg) [3]. All objects were introduced intragastrically one hour before the experiment. Acute hypobaric hypoxia was modeled by «lifting» mice in a hyperbaric chamber (h =11000 m, $\upsilon$=100 m/s) [10]. All findings were processed by means of variation statistics methods using the STATISTICA 6.0 software.

## RESULTS

When hypobaric hypoxia was reproduced, the life time of the NC group animals was 28,9 ± 1,62 sec. When using Metaprot, the life expectancy of animals exceeded the analogous indices of the NC group of mice under conditions of hypobaric hypoxia by 2,2 times ($p < 0,05$). Testing for the resistance of mice to acute hypobaric hypoxia showed that the average lifespan of animals receiving the experimental VMA-10-18 derivative intragastally was 78,3 ± 1,83 seconds, which was 2,7 times ($p < 0,05$) statistically significantly higher than the control group.

At the same time, the life expectancy of animals receiving VMA-10-18 exceeded the value of the Metaprot group by 1,2 ($p < 0,05$), which was statistically significant.

## CONCLUSION

The use of a new derivative VMA-10-18 (10 mg/kg) significantly increased the lifespan of mice:

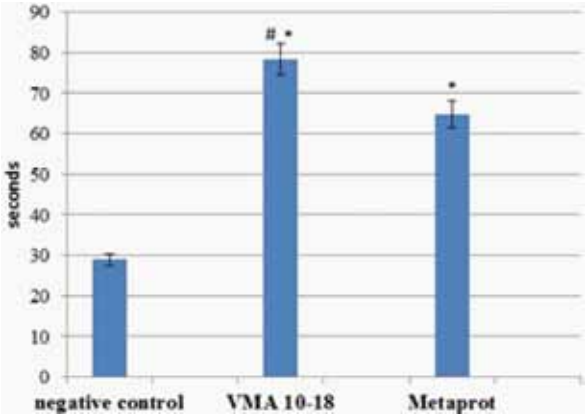


***Fig. 1.*** *Assessment of the effect of the new derivative VMA-10-18 and Metaprot on the lifespan of mice under hypobaric hypoxia*
***Note:*** *Control — a control group of mice, Metaprot — a group of mice that received Metaprot; VMA-10-18 — a group of mice treated with VMA-10-18; * — statistically significant for the control group of mice (p <0,05); # — statistically significant for the group of mice treated with Metaprot (p <0,05).*

by 2.2 times compared to the control group and 1,2 times versus the mice receiving Metaprot. The outcomes may indicate the antihypoxic effect of this compound exceeds that of the reference drug Metaprot at a dose of 25 mg/kg. Since the antihypoxic effect can be one of the mechanisms of actoprotective activity, this compound is a promising object for further study and correction of mental and physical performance.

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# EFFECTS OF GLYPROLINES ON THE BEHAVIOR OF RATS IN THE PORSOLT TEST AND EXPERIMENTALLY INDUCED SOCIAL STRESS

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**ABSTRACT** — In this work we studied the effect of glyprolines on the behavior of rats under conditions of experimental social stress. White male rats 6-8 months of age were used in the experiment. Throughout the experiment all animals were kept in standard-barrier conditions. The effect of glyprolines on the behavior of white male rats was studied on the model of social stress, implying that the animals are kept in conditions of a constant sensory contact. Glyprolines (Selank, Pro-Gly-Pro, Pro-Gly-Pro-Leu) were injected to the animals Intraperitoneally at a dose of 100 μg/kg/day within 20 days. Porsolt test was employed to carry out behavioral analysis in the animals. The outcomes revealed that regardless of the type of behavior (aggressive and submissive) in all the animals depression and anxiety disorders were developed, whereas Selank, Pro-Gly-Pro, Pro-Gly-Pro-Leu facilitated their alleviation. Thus, our experiment has demonstrated a psychomodulatory effect of the glyprolines.

**KEYWORDS** — experimental social stress, neuropeptides, glyprolynes, Selank, Pro-Gly-Pro, Pro-Gly-Pro-Leu, psychomodulatory effect.

## INTRODUCTION

Recent studies have given evidence that any stress including a social stress may cause disruptions of adaptive mechanisms, which lead to occurrence of pathological symptoms and adaptive problems [1, 9]. Stress-induced disturbances can emerge at any stage of the stress response and affect all major systems of the body which may account for the use of pharmacological correction [8, 11].

To date, neuropeptides are often used as a basis for the development of drugs with a stress-protective effect which can have a multifaceted effect on the body as a whole [2, 5]. Neuropeptides are highly active due to their natural origin and unique structure with little or no toxicity. Currently, neuropeptides are mostly represented by drug Selank which is synthesized at the Kurchatov Institute of Molecular Genetics. It has been found that Selank has antioxidant, antihypoxic, immunotropic and other activities [3, 10].

The anxiolytic, antistressor, nootropic and other properties of this drug have been confirmed in various studies [6, 7]. Despite accumulated data from the clinical studies on the spectrum of their pharmacological activities, the properties of new neuropeptide compounds have been actively researched.

*The aim of the work* was to study the effect of neuropeptides on the behavior of male rats exposed to experimental social stress in the Porsolt test.

## MATERIAL AND METHODS

The study was conducted on 90 male white rats of 6 months of age. All manipulations with rats were conducted in compliance with the DIRECTIVE 2010/63/EU on the protection of animals used for scientific purposes. All animals were held in standard vivarium cages during the experiment. The study of the effect of neuropeptides on the behavior of white male rats was carried out on the model of social stress. Its methodological requirement is that animals are constantly kept in conditions of sensory contact [4]. The animals were housed in pairs in cages with transparent partitions with holes that provide sensory contact when social stress was formed. Every day the partition was removed for 10 minutes which led to inter-male confrontations as a result of which groups were formed: aggressor rats, whose behavior was manifested in the form of vertical and lateral stands (*threat*) or attacks; prey rats, the submissiveness of which manifested itself in the form of locomotion, sniffing, autogrooming, movements in place, vertical *protective* stands, immobility.

All animals were divided into groups: intact males (control); animals exposed to social stress (stress); and experimental groups who received intraperitoneal injections of neuropeptides (Selank, Pro-Gly-Pro, Pro-Gly-Pro-Leu) at a dose of 100 μg/kg/day for 20 days starting from the 1st day of the experiment.

The effect of neuropeptides on the psychoemotional state of white rats exposed to social stress was evaluated based on the study of animal behavior in the Porsolt test.

The experiment results were statistically processed using the following programs: Microsoft Office Excel 2007 (Microsoft, USA), BIOSTAT 2008 Professional 5.1.3.1. To process the obtained results we used a parametric method of the Student t-test with the Bonferroni correction. Statistically significant difference was considered at p.0.05.

## RESULTS

The results obtained at the end of Porsolt test indicate the formation of anxiety-depressive state in animals, which was expressed in an increase of the general period of immobility in aggressors and victims compared with the control group by 35% (p<0.05) and 63% (p<0.01); an increase in the time of passive swimming by 58% (p) 0.01) and 70% (p<0.001); a decrease in the time of active swimming by 34% (p<0.05) and 45% (p<0.05) respectively.

Under the influence of the investigated neuropeptides the changes were observed that confirm a corrective effect of these compounds on the psychoemotional state of white rats. So it was found that the latency period before the first movement in comparison with the stress group decreased in the groups of both aggressors and victims: Selank — by 29% (p<0.05) and 38% (p<0.01), Pro- Gly-Pro — by 25% (p<0.05) and 38% (p<0.05), Pro-Gly-Pro-Leu — by 32% (p<0.05) and 25% (p<0.05) respectively (Fig. 1).

There was also a statistically significant increase in the latency period to the first immobility in all groups of rats in comparison with the stressed period: Selank contributed to the increase in the period by 25% (p>0.05) and 36% (p<0.05), Pro-Gly-Pro — by more than 30% (p<0.05), Pro-Gly-Pro-Leu — by 22% (p>0.05) and 30% (p<0.05) (Fig. 2).

The introduction of preparations of neuropeptide analogs led to a statistically significant change in the time of immobility of animals during passive swimming in the groups of aggressors and victims in comparison with the stressed group of rats: under the influence of Selank — by 27% (p<0.05) and 32% (p<0.05 ), Pro-Gly-Pro — 31% (p<0.05) and 44% (p<0.01); Pro-Gly-Pro-Leu — 29% (p<0.05) and 26% (p<0.01), respectively (Fig. 3).

The time of passive swimming also decreased in comparison with the stress group in animals with aggressive and submissive types of behavior: Selank — by 35% (p<0.01) and 42% (p<0.01), Pro-Gly-Pro — by 37% (p<0.01) and 34% (p<0.05) respectively, Pro-Gly-Pro-Leu — by 38% on average (p<0.01) (Fig. 4).

The time of active swimming with the introduction of neuropeptides increased in comparison with the stressed control, both in the group of animals with aggressive and submissive types of behavior. So the introduction of Selank contributed to the increase in this indicator by 54% (p<0.01) and 79% (p<0.001), Pro-Gly-Pro — by 55% (p<0.01) and 79% (p<0, 01), Pro-Gly-Pro-Leu — by 52% (p<0.01) and 71% (p<0.01) respectively (Fig. 5).

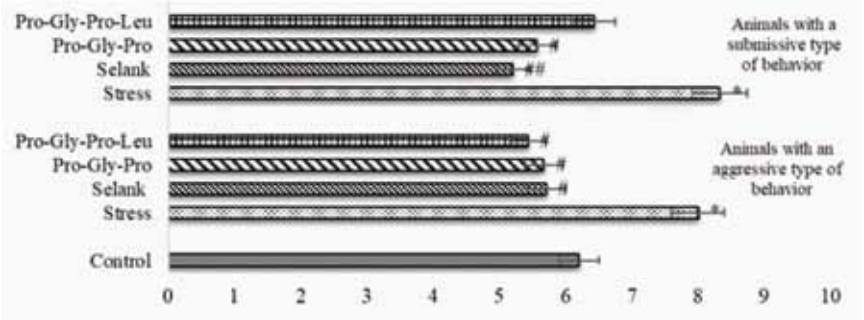


***Fig. 1.*** *Duration of the latent period before the first movement of animals in the Porsolt test*
***Note:*** ** — p <0.05; ** — p <0.01; *** — p <0.001 — relative to the control; # — p <0.05; ## — p <0.01; ### — p <0.001 — relative to stressed animals (Student's t-test)*

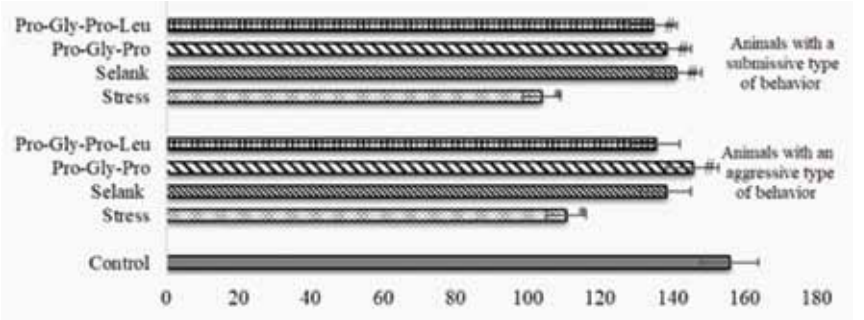


***Fig. 2.*** *Duration of the latent period before the first immobility of animals in the Porsolt test*
***Note:*** ** — p <0.05; ** — p <0.01; *** — p <0.001 — relative to the control; # — p <0.05; ## — p <0.01; ### — p <0.001 — relative to stressed animals (Student's t-test)*

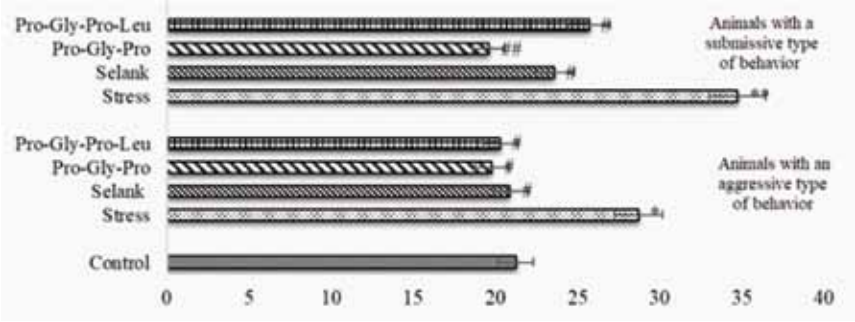


***Fig. 3.*** *Duration of animal immobility in the Porsolt test*
***Note:*** ** — p <0.05; ** — p <0.01; *** — p <0.001 — relative to the control; # — p <0.05; ## — p <0.01; ### - p <0.001 — relative to stressed animals (Student's t-test)*

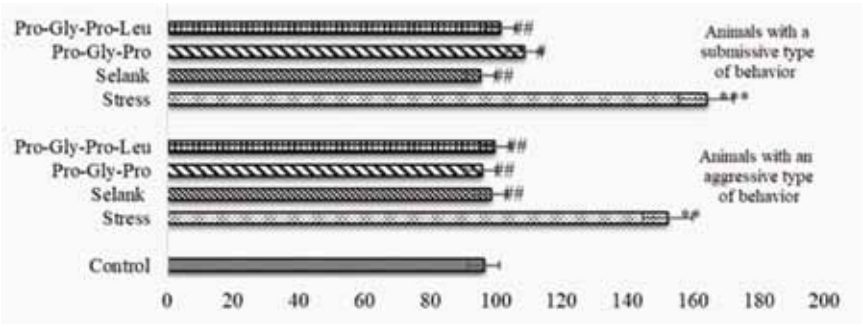


***Fig. 4.*** *Duration of passive swimming of animals in the Porsolt test*
***Note:*** ** — p <0.05; ** — p <0.01; *** — p <0.001 — relative to the control; # — p <0.05; ## — p <0.01; ### — p <0.001 — relative to stressed animals (Student's t-test)*

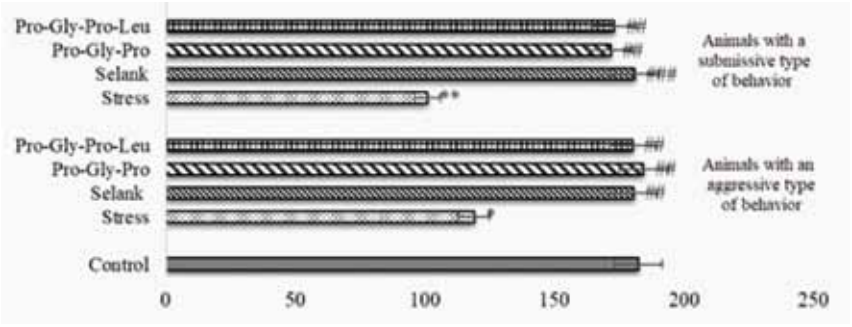


***Fig. 5.*** *Duration of active swimming of animals in the "Porsolt" test*
***Note:*** ** — p <0.05; ** — p <0.01; *** — p <0.001 — relative to the control; # — p <0.05; ## — p <0.01; ### — p <0.001 — relative to stressed animals (Student's t-test)*

## CONCLUSION

The outcomes of our study on the behavior of white male rats in the Porsolt test provide evidence that the intraperitoneal administration of glyprolines (Selank, Pro-Gly-Pro, Pro-Gly-Pro-Leu) helps to reduce increased anxiety levels in animals both with aggressive and submissive types of behavior because of their psychomodulatory effects.

### *Conflict of Interest*

Authors have no conflict of interest to declare

### *Funding*

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### *Compliance with Ethical Standards*

The research protocol was approved by the Ethic Committed of Astrakhan State Medical University No 8 from 24.11.2015

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# EFFECTS OF MELANOCORTINSON THE BEHAVIOR OF RATS IN THE TEST OF ELEVATED CRUCIFORM MAZE AND EXPERIMENTALLY INDUCED OF SOCIAL STRESS

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**ABSTRACT** — In this study we have investigated the effects of melanocortins (group analogs of neuropeptides) on the psychoemotional state of rats under conditions of experimental social stress. The study was carried out on male rats which were injected intraperitoneally for 20 days with the neuropeptide drugs Semax and ACTH(6-9)-Pro-Gly-Pro at a dose of 100 μg/kg/day starting from the 1st day of stress exposure. The psychoemotional state of the animals was evaluated by means of the behavioral test of elevated cruciform maze. It was found that intraperitoneal administration of neuropeptides (Semax, ACTH(6-9)-Pro-Gly-Pro) helps to reduce high anxiety levels in animals both with aggressive and submissive types of behavior under conditions of experimental social stress via their psychomodulatory effects.

**KEYWORDS** — experimental social stress, neuropeptides, melanocortins, Semax,ACTH(6-9)-Pro-Gly-Pro, psychomodulatory effect.

## INTRODUCTION

Recent studies indicate that a variety of stressful influences play an important role in the development of depressive disorders [1, 2, 3]. The modern society with its accelerated pace of life, the lack of physical activity, lack of time facilitate the development of stress-related conditions which act as risk factors for both mental and somatic disorders. [1, 7]. Therefore, the issues of timely prevention and correction of stress and possible solutions how to cope with its clinical outcomes have remained relevant. There is a promising trend to use for correction of pathological conditions of the nervous system drugs from the group of analogs of neuropeptides which are similar to natural endogenous regulators of the body functions. [4, 5, 6, 8]. Melanocortins are synthesized at the Kurchatov Institute of Molecular Genetics (Moscow, Russia). The registered drug Semax (ACTH(4-7)-Pro-Gly-Pro) and the new compound ACTH(6-9)-Pro-Gly-Pro are of special interest due to their potency of compensating for stress-related damage.

*The aim of research* is to study the effect of neuropeptides/melanocortins on the psychoemotional state of white male rats under conditions of experimental social stress.

## MATERIAL AND METHODS

The study was carried out on 70 white male rats. All manipulations with rats were conducted in compliance with the DIRECTIVE 2010/63/EU on the protection of animals used for scientific purposes. The animals were divided into groups (n=10): group 1 — intact animals (control); group 2 — rats exposed to social stress for 20 days and 2 groups of experimental animals exposed to social stress and receiving neuropeptides at doses of 100 mg/kg per day intraperitoneally for 20 days: registered drug Semax and new compound ACTH(6-9)-Pro-Gly-Pro. In our experimental model of social stress rats were paired-housed in cages separated by a transparent partition with holes. The rats were able to see, hear and smell each other, but at the same time the partition prevented from physical interaction. The septum was removed for 10 minutes every day, resulting in inter-male confrontation. As a result, groups of animals with aggressive and submissive behaviors were formed. The anxiety-related behavior of the rodents was assessed by using the test of elevated cruciform maze.

The experiment results were statistically processed using the following programs: Microsoft Office Excel 2007 (Microsoft, USA), BIOSTAT 2008 Professional 5.1.3.1. To process the obtained results, a parametric method was used with the Student t-test with the Bonferroni correction. Statistically significant difference were considered at p 0.05.

## RESULTS

Modeling of experimental social stress on male rats led to similar changes in behavioral indicators in groups of animals with aggressive and submissive

behaviors indicating the formation of high anxiety which manifested itself as a decrease in the number of stands by more than 45% ($p < 0.05$), the number of exits into the open sleeves of test by 45% ($p < 0.05$) and 35% ($p<0.05$); reducing the time spent on open sleeves by 32% ($p < 0.05$) and 40% ($p < 0.05$); decrease in the number of exits to the center by 47% ($p < 0.05$) and 30% ($p > 0.05$); a decrease in the time spent in the center by 36% ($p < 0.05$) and 45% ($p < 0.001$) in the groups of aggressors and victims respectively in comparison with intact animals.

There was also a statistically significant increase in the number of visits to the closed sleeves and the number of fecal boluses by almost 100%, the number of *peeking* from the closed sleeves by almost 50% in groups of rats with both aggressive and submissive behaviors compared to control animals.

The results of experiment in the test of elevated cruciform maze showed that the neuropeptides were responsible for similar changes against the background of social stress: the time spent on open arms increased significantly by more than 40% ($p<0.05$) in comparison with the stressed group of rats (Fig. 1).

Under the influence of neuropeptides, the time spent in the center increased by 30% ($p<0.05$) in the group of aggressors, by 50% ($p<0.01$) in the group of animals with submissive behavior (Fig. 2).

Statistically significant changes were observed in the aggressors and victims when counting the number of *peeking* from the closed sleeves: Semax contributed to a decrease in the indicator by 2 ($p<0.001$) and 1.3 ($p<0.05$) times respectively; ACTH(6-9)-Pro-Gly-Pro — 2.4 ($p<0.001$) and 1.3 ($p<0.05$) times respectively (Fig. 3).

The number of stands in comparison with the stress group under the administration of the studied compounds increased in the groups of animals with aggressive and submissive types of behavior: under the influence of Semax by 1.9 ($p<0.01$) times; ACTH (6-9)-Pro-Gly-Pro — 1.9 ($p<0.01$) and 1.8 ($p<0.001$) times respectively (Fig. 4).

Due to the use of neuropeptides a statistically significant decrease in the number of fecal boluses was noted in comparison with the stress group of both aggressive rats and the group with submissive behavior: Semax caused a decrease in 4.5 ($p<0.001$) and

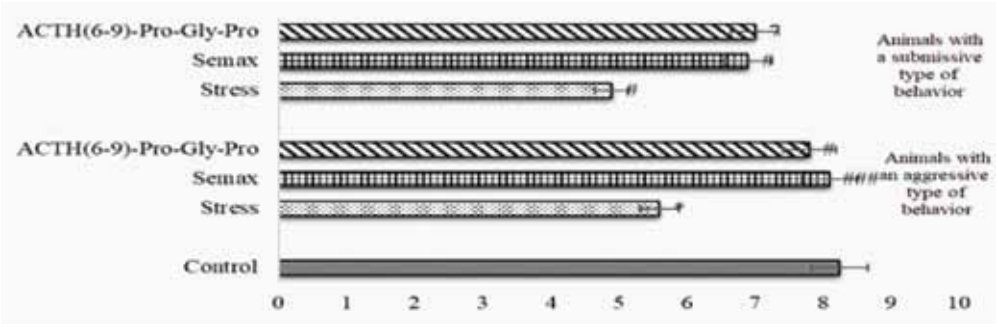


***Fig. 1.*** *Time spent by animals on open sleeves in the test of elevated cruciform maze*
***Note:*** ** — p <0.05; ** — p <0.01; *** — p <0.001 — relative to the control; # — p <0.05; ## — p <0.01; ### — p <0.001 — relative to stressed animals (Student's t-test)*

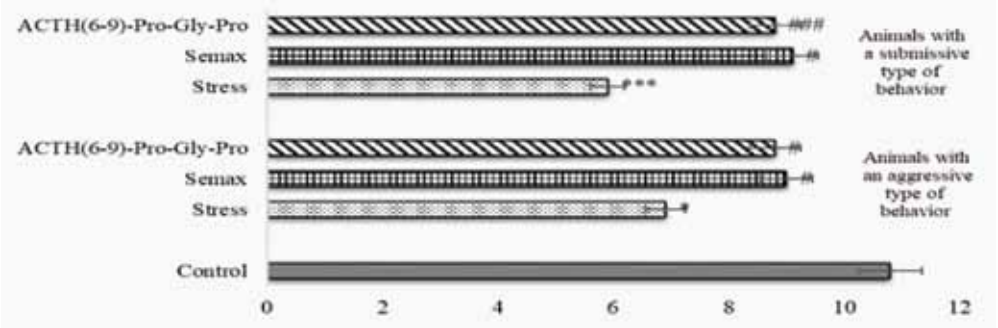


***Fig. 2.*** *Time spent by animals in the center area of the elevated cruciform maze.*
***Note:*** ** — p <0.05; ** — p <0.01; *** — p <0.001 — relative to the control; # — p <0.05; ## — p <0.01; ### — p <0.001 — relative to stressed animals (Student's t-test)*

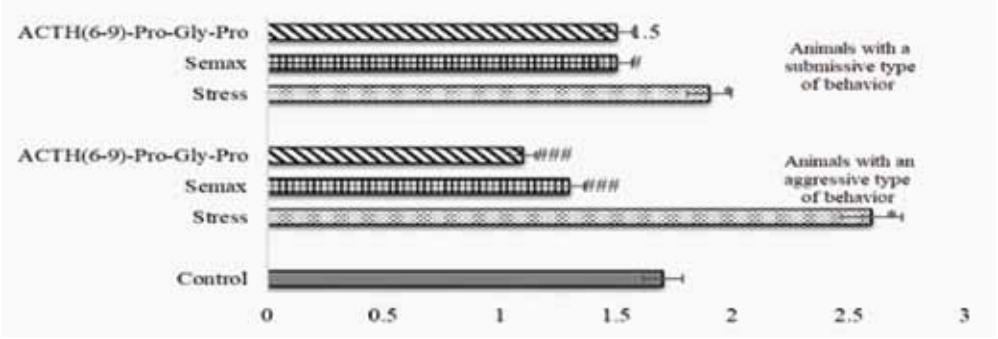


***Fig. 3.*** *Number of "peeking" from closed sleeves of the Elevated cruciform maze*
***Note:*** ** — p <0.05; ** — p <0.01; *** — p <0.001 — relative to the control; # — p <0.05; ## — p <0.01; ### — p <0.001 — relative to stressed animals (Student's t-test)*

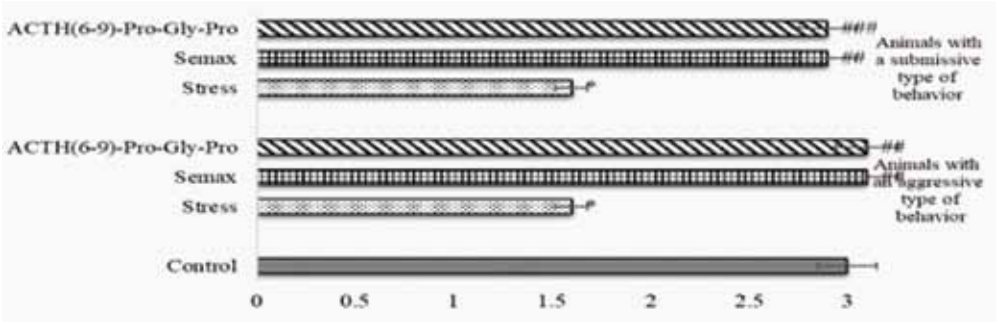


***Fig. 4.*** *Number of stands of animals in the test «Elevated cruciform maze»*
***Note:*** ** — p <0.05; ** — p <0.01; *** — p <0.001 — relative to the control; # — p <0.05; ## — p <0.01; ### — p <0.001 — relative to stressed animals (Student's t-test)*

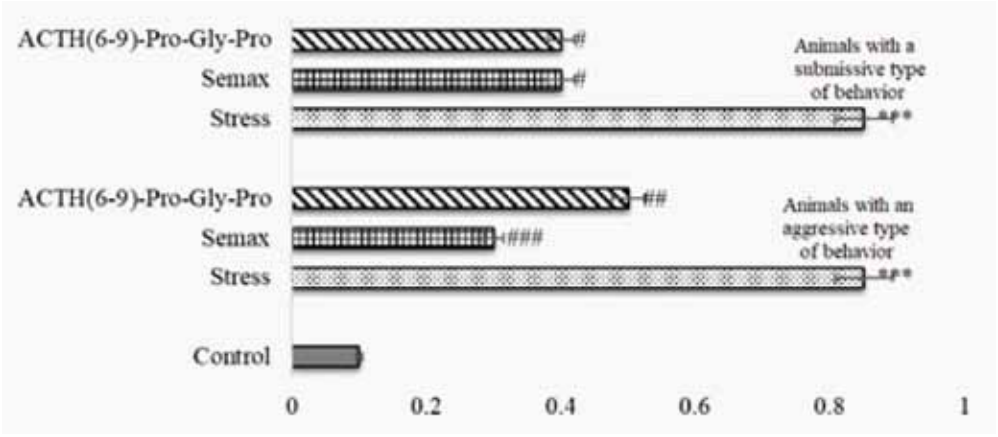


***Fig. 5.*** *Number of fecal boluses in the test «Elevated cruciform maze»*
***Note:*** ** — p <0.05; ** — p <0.01; *** — p <0.001 — relative to the control; # — p <0.05; ## - p <0.01; ### — p <0.001 — relative to stressed animals (Student's t-test)*

2.6 ($p<0.05$) times; ACTH(6-9)-Pro-Gly-Pro — 2.3 ($p<0.01$) and 2.7 ($p<0.05$) times respectively (Fig. 5).

However, less pronounced changes were observed when evaluating the indicators: *visiting open sleeves*, *visiting closed sleeves* and *exits to the center* and were not statistically significant in all cases.

## CONCLUSION

The outcomes of the study of the behavior of white male rats in the tests of elevated cruciform maze allow us to conclude that the use of of neuropeptides (Semax, ACTH(6-9)-Pro-Gly-Pro) due to their psychomodulatory effects help to reduce anxiety in animals both with aggressive and submissive types of behavior in a model of social stress.

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# HEMODYNAMICS STATE IN STUDENTS OF MEGAPOLIS UNIVERSITIES: SINGLE-CENTER COHORT STUDY

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**ABSTRACT** — The purpose of this study was to estimate hemodynamics parameters in students during an inter-sessional period. The study included 208 students from medical university in the large metropolis (Nizhny Novgorod, Russia). It was found that this group of population has good adaptive reserves. However, in less than 20% of examined students we may suggest signs of sympathicotonia and a moderate risk of arrhythmogenic events.

**KEYWORDS** — students, hemodynamics, heart rate variability, microcirculation.

## INTRODUCTION

In the light of modern trends in the formation of the population's commitment to physical training and a healthy lifestyle, the willingness of people to follow them, that is, to maintain the level of functional reserves of their body, comes to the fore [1, 2]. In this connection, comprehensive monitoring of the health status of the population is necessary, which is partially implemented through the medical examination procedure [2, 3, 8]. It is well-known that students are the part of the population that is most actively involved in the training process and high physical activity (within the framework of sports activities, physical training and a healthy lifestyle) — [1, 3, 5–8]. Therefore, we have assumed that the assessment of their adaptive capacity should preferably be made on the basis of analysis of the cardiovascular system. It is well-known that this system is most responsive to shifts of homeostasis and change settings for environment exposures [1–8].

Based on our assumption the ***purpose of this study*** was to study the state of hemodynamics parameters in students of the medical university of Nizhny Novgorod during the inter-sessional period.

## MATERIAL AND METHODS

The study included 208 students (18–20 years old; 76/132 as male/female) from the medical university of a large metropolis (Nizhny Novgorod, Russia). The study was conducted in the middle of the day, in a calm state (during the inter-session period, outside the days of passing tests or colloquiums) in full compliance with the standard rules of procedure for taking an electrocardiogram (ECG). To register ECG and analyze hemodynamic parameters, including those that characterize heart rate variability, we used the "Medical Soft" sports testing system (MS FIT Pro version, Russia). Standard hemodynamic parameters (blood pressure level, pulse rate, stroke volume, cardiac output, etc.), statistical and spectral parameters of heart rate variability, as well as an integral criterion of the state of microcirculation were used for monitoring. Data analysis was performed in accordance with age standards formed by equipment developers based on age standards [3–6].

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

## RESULTS

Analysis of the main parameters of systemic hemodynamics allowed us to form a comprehensive view of the state of the cardiovascular system in students of the medical university of Nizhny Novgorod (Table 1).

It was found that the level of blood pressure (both systolic and diastolic) of the considered contingent of students does not differ from the age norm, while the average heart rate is at the upper limit of the physiological range, which indicates a tendency to moderate tachycardia. At the same time, other indicators that characterize the pumping function of the heart (stroke volume, cardiac output) remain within the normal range. This indicates the adaptive character of the observed level of pulse rate.

The contribution of peripheral vascular resistance to the formation of systemic blood flow was evaluated by calculating the corresponding parameter, which was also recorded at the upper limit of the age norm. On the other hand, the arterial stiffness index, which indicates the state of the vascular wall, is also determined in the physiological range, which allows us to interpret

***Table 1.*** *Hemodynamics parameters in students*

| Parameter | Value (M±m) | Age norm |
|---|---|---|
| Systolic pressure, mm.Hg. | 121,33±2,94 | 110-140 |
| Diastolic pressure, mm.Hg. | 71,50±2,91 | 75-90 |
| Pulse rate, min-1 | 87,58±2,72 | 70-90 |
| Stroke volume, ml | 65,19±1,97 | 60-90 |
| Cardiac output, l/min | 5,60±0,19 | higher than 4,5 |
| Total peripheral resistance, rel. un. | 1290,94±35,42 | lesser than 1300 |
| pNN50, % | 30,66±1,07 | 10-49 |
| Spectral balance index (LF/HF), rel. un. | 1,29±0,08 | lesser than 2,0 |
| Stress index, points | 7,38±0,09 | 8-10 |
| Arterial stiffness, points | 9,89±0,12 | 8-10 |
| Microcirculation, points | 9,50±0,18 | 8-10 |

the identified trend as adaptive, which may be due to the peculiarities of the hormonal background of students and an increased number of stressful situations that induce activation of the sympatho-adrenal system. This mechanism is additionally indicated by the level of the stress index, which shifts towards disadaptation and goes beyond the optimal age limit.

Evaluation of the parameters of heart rate variability allowed us to establish the presence of relative instability of hemodynamic support, as evidenced by a fairly high value of the pNN50 indicator (30.66%). Despite falling within the physiological range, this indirectly characterizes the cardiac rhythm in the considered group of individuals as highly variable, which is a predictor of an increased risk of arrhythmogenic events. At the same time, a positive fact in the assessment of heart rate variability in the examined individuals is the finding of the spectral balance index (LF/HF), calculated on the basis of spectral analysis of the heart rate variability, in the age range corresponding to normotonia. Only less than 20% of the students included in the study had signs of sympathicotonia for this indicator. This provides adequate conditions for ensuring blood flow through the microvascular bed, which is reflected in the physiological level of the microcirculation.

## CONCLUSION

Using comprehensive hardware testing of the state of the cardiovascular system of medical students in Nizhny Novgorod, it was found that this population group has good adaptive reserves, but some of the examined students showed signs of sympathicotonia and a moderate risk of arrhythmogenic events.

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# CONCERNING SOME MORPHOFUNCTIONAL ASPECTS OF THE UTERINE CERVICAL RIPENENING

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**ABSTRACT** — The onset of pregnancy is marked by profound morphological and functional changes occurring in the uterine cervix, the whole combination of which is described by the term *cervical ripenening*. The study of morphological and functional aspects of cervical maturation is both of theoretical and practical importance since many disorders of cervical maturation often lead to premature delivery or the birth of premature infants. The improvement of research methods enabled to study the process of cervical maturation at the molecular and cellular levels of organization. Our paper summarizes current data on the main morphological and functional changes that occur in the uterine cervix during maturation. In the course of the analysis of literature data, it became possible to identify three structural components that play a leading role in the maturation of the uterine cervix: epithelial, immune-inflammatory, connective tissue extracellular matrix component. Understanding the morphofunctional changes that occur in the uterine cervix during its maturation at different levels of organization of living organisms (molecular, cellular, tissue) is crucial for the development and improvement of treatment and prevention strategies.

**KEYWORDS** — Uterine cervix, pregnancy, maturation, premature delivery, epithelium, inflammation, extracellular matrix.

## INTRODUCTION

The uterine cervix is a structure located at the caudal end of the uterus. Its main function is to keep the fetus in the uterus during pregnancy until the onset of labor. The structure of the uterine cervix is represented by the most important components like epithelial tissue, smooth muscle cells, as well as connective tissue, including fibroblast cells and extracellular matrix. With the onset of pregnancy, numerous ultrastructural changes arise in these components of the uterine cervix, the study of which is an important research area that has not only theoretical but also practical importance [14–18, 38]. Thus, the optimal course of the process of the uterine cervix maturation is a prerequisite for a successful natural delivery. Accordingly, a violation of the process of the uterine cervix maturation and its premature dilatation can lead to preterm delivery, which is an urgent problem of modern healthcare. According to statistics, 12.5% of all pregnancies, where any abnormality occur during the maturation of the uterine cervix, end in premature delivery [15, 16]. In this regard, studies aimed at establishing fundamental morphofunctional changes occurring during cervical maturation are essential for the prevention of early delivery and the birth of premature infants.

*The purpose of this article* is to discuss the main morphological and functional changes that occur in the uterine cervix during maturation. Below, we will sequentially review the role of cervical epithelial cells, components of the extracellular matrix of its connective-tissue stroma as well as immune-inflammatory factors and cells that are activated and/or migrate into the uterine cervix during pregnancy, ensuring the process of its maturation.

## ROLE OF THE EPITHELIAL CELLS IN THE UTERINE CERVIX MATURATION

The mucous membrane of the uterine cervix, consisting of the epithelium and the lamina propria, plays an important protective role during pregnancy and delivery, which is to prevent the penetration of infectious agents into the uterine cavity and mechanical damage during the passage of the fetus during labor. It was noted that during pregnancy, epithelial cells actively multiply and differentiate [16, 42, 46]. Differentiation of epithelial cells is accompanied by huge changes in the expression of regulatory genes and products of their transcription (messenger RNA) and translation (protein and enzyme molecules). Due to the improvement of molecular genetic research methods and a series of experiments to study the expression of specific compounds in the epithelial tissue of the uterine cervix, it became possible to obtain interesting data. So, for example, in studies, significant alteration in the expression of aquaporins, fissural junction proteins (contacts) — connexin 26 and 43, enzymes of hyaluronan synthase 2, steroid 5-alpha-reductase type 1

and desmogleins 1α and 1β were found [4, 32, 37, 42]. The data from these studies clearly indicate that during pregnancy there is a carefully regulated expression of specific proteins and enzymes that are necessary to optimize the process of cervical maturation.

Epithelial tissue of the female reproductive tract, including the uterine cervix, is essential in providing protective reactions of innate and acquired immunity, aimed at preventing the development of ascending infection. It has been established that epithelial cells secrete cytokines and chemokines that recruit and activate immune-inflammatory cells and antimicrobial factors that destroy invading pathogens [45]. An increased number of leukocytes is found in the cervical lamina propria in mice during pregnancy, which is recruited by some cytokines and chemokines for immunological surveillance. Additionally, epithelial cells themselves also produce bacterial and viral pattern recognition receptors (Toll-like receptors, antimicrobial factors, and protease inhibitors). Y. Filipovich and colleagues, using an experimental model of preterm labor (induced by infectious agents) in mice, established the important role of the adapter protein MyD88 (TIRAP (toll-interleukin 1 receptor (TIR) domain containing adaptor protein)) in the initiation of preterm delivery. In mice deficient in MyD88, preterm labor did not develop in response to infectious agents [11, 12]. Further study of this mechanism, according to the researchers, is promising in terms of the development of therapeutic and prophylactic medications for the prevention of preterm labor by inhibiting MyD88 and intracellular signaling pathways.

The regulation of the epithelium barrier properties of the uterine cervix mucous membrane is ensured by protein molecules of tight junctions, which, under normal conditions, reliably isolate the spaces between adjacent epithelial cells. According to the results of an experimental study, in mice during pregnancy, there are changes in the content and structure of protein molecules of tight junctions, in particular, in claudin 1 and 2 [43]. Apart from the aforesaid, the expression of desmoglein and a number of keratin proteins increases during cervical maturation and becomes maximal during delivery [13, 43]. The specific functions of tight junction proteins of cervical epithelial cells and their role in epithelial cell differentiation and cervical maturation still remain unclear. However, changes in expression in animals during experimental modeling of preterm birth may indicate that the permeability and, accordingly, the barrier properties of the epithelium are regulated during pregnancy and play an important role in the maturation of the uterine cervix [13, 43, 47].

Rather interesting are reports of additional proteins expressed in the cervical mucosa, such as trefoil factor 1 (TFF1) and the serine protease inhibitor Kazal type 5 (SPINK5/LEKT1). The expression of these proteins increases during cervical maturation and becomes maximal during delivery [9, 25]. The important functions of TFF1 and SPINK5 may be indirectly evidenced by studies on other organs. For example, it has been shown that TFF1 plays an important role in the protection and restoration of the epithelial layer of the gastric mucosa and small intestine [25]. SPINK5 is also expressed in the epithelial layer of the skin and prevents the degradation of desmogleins and some other proteins involved in the formation of the protective barrier of the epithelium [9]. Based on the functions of TFF1 and SPINK5 proteins in the organs of the gastrointestinal tract and skin, it can be assumed that their increased expression in the mucous membrane of the cervix during maturation is also not accidental and, probably, supports the protective properties of the uterine cervix of pregnant women to fight infectious pathogens. In general, a decrease or absence of expression of protective factors can contribute to an increase in susceptibility to infectious-mediated premature birth, and therefore, further study of such mechanisms seems to be a promising research direction.

## ROLE OF IMMUNE AND INFLAMMATORY COMPONENTS IN THE UTERINE CERVIX MATURATION

According to one hypothesis, immune and inflammatory cells cause changes in the structure and properties of the uterine cervix extracellular matrix, which can lead to its early maturation and preterm birth [24, 31]. As a result of observations, it was suggested that leukocytes infiltrating the uterine cervix by the time of delivery secrete protease enzymes that contribute to the destruction, loss, and disorganization of the extracellular matrix rich in collagen, which leads to the expansion of the uterine cervix [24, 26, 31, 35, 42, 48] Studies have shown that inflammatory cells (neutrophils, eosinophils, macrophages of the M1 and M2 phenotypes, and others), secreting pro-inflammatory cytokines, are widely represented in the uterine cervix before delivery, both in women and in experimental animals [33, 41]. Also, in the postpartum period, there is a sharp change in the expression profile of macrophage genes. As early as two hours after birth, in mice, M1 macrophages express classical pro-inflammatory markers: interleukin 1 alpha, tumor necrosis factor-alpha (TNF-alpha), monocytic chemotactic protein 1. Alternatively, activated (anti-inflammatory) M2 macrophages express the following markers: chitinase-3-like protein, arginase-1, is an antagonist of the interleukin 1 receptor, which

takes on enormous importance in the immunosuppression and repair of organs and tissues, including the uterine cervix [33, 41, 42]. These data support the theory of preterm labor mediated by infectious and inflammatory processes.

Macrophages have a heterogeneous phenotype, the change of which at different stages of cervical maturation plays a role in creating optimal conditions for pregnancy and facilitating the effective recovery of the uterine cervix after delivery. In general, a mixed population of macrophages serves two main purposes: 1) macrophages of the M1 phenotype protect the reproductive tract from the threat of microbial invasion and provide postpartal clearance of extracellular matrix molecules necessary during cervical maturation, and 2) macrophages of the M2 phenotype suppress hyperactivation of pro-inflammatory reactions and promote restoration of the structure of the uterine cervix to its original (non-pregnant) state, which is necessary after childbirth [33, 41].

It is also notable that the role of inflammation in cervical maturation, according to a series of studies, can be controversial. Some studies have reported a slight increase in the expression of genes encoding pro-inflammatory proteins during cervical maturation [19]. In another study, on the contrary, there was a significant increase in the expression of proinflammatory factors in the uterine cervix during maturation and during vaginal delivery [19, 20]. Many researchers believe that infections and inflammatory responses are triggers for premature cervical maturation, significantly increasing the likelihood of preterm birth. Thus, according to some data, infection-mediated premature deliveries account for approximately 25–40% of all causes of preterm births [6, 15].

The ability of inflammation to induce preterm labor is clearly demonstrated by an experimental study in which the administration of a Toll-like receptor 4 antagonist to non-human primates reduces the risk of preterm labor caused by infectious pathogens [1]. In another experimental study, it was shown that mice with a deficiency of receptors for pro-inflammatory markers (interleukin 1-alpha and tumor necrosis factor-alpha) are more resistant to preterm labor induced by exposure to one of the key pathogenicity factors of gram-negative microbes — lipopolysaccharide (LPS). LPS is very often used for experimental modeling of preterm delivery in animals [21, 22].

## ROLE OF EXTRACELLULAR MATRIX COMPONENTS IN THE UTERINE CERVIX MATURATION

The components of the extracellular matrix, due to their high content in the uterine cervix, make an invaluable contribution to the process of its maturation. Changes in the uterine cervix, characterized by its significant softening in women in early pregnancy, were described by the German researcher A. Hegar back in 1895. The results of these initial studies provided the first evidence for structural changes occurring in the uterine cervix during the first trimester of pregnancy, characterized by increased cervical compliance in response to mechanical stress [10]. A subsequent more detailed study of the biomechanical properties of the uterine cervix at different stages of pregnancy confirmed that the compliance or distensibility of the uterine cervix increases in the early stages of pregnancy and becomes maximal by the time of delivery [10, 23]. Changes in the biomechanical properties of the uterine cervix play a significant role in maintaining a normal pregnancy [10].

The development of research methods made it possible to establish that the biomechanical properties of the uterine cervix are closely related to its structural components, among which the proteins of the extracellular matrix should be specially noted [14–16, 29, 30, 40]. Collagen is the most abundant protein in the entire human body, including individual organs such as the uterine cervix. Fibrillar collagen is considered to be the main structural protein that affects such biomechanical properties of the uterine cervix as strength and distensibility [14–16]. The properties of collagen are partially affected by changes in its synthesis, post-translational (post-synthetic) modifications (folding, glycosylation, etc.), assembly of synthesized collagen chains into fibers, and degradation of its fibers. In the literature, there are conflicting data on the role of collagen in cervical maturation [14–16, 29, 30, 40]. Some researchers believe that different types of collagen significantly affect the biomechanical properties of the uterine cervix as it matures. There is an opinion, that this assumption is the most convincing. So, in the non-pregnant uterine cervix and the early stages of its maturation (pregnancy), type 1 collagen prevails. It determines the increased stiffness of the uterine cervix and got cleaved by proteases when the uterine cervix is mature. At the same time, along with the processes of the breakdown of type 1 collagen during the maturation of the uterine cervix, there is an increase in the synthesis of type 3 collagen, the functional properties of which differ from type 1 collagen. Based on statistical analysis, a positive correlation was noted between type 3 collagen synthesis and gestational age. The maximum content of type 3 collagen is observed at birth and, according to the previous studies, is associated with an increase in the distensibility of the uterine cervix [15, 16]. Consequently, different types of collagen, changing during the maturation of

the uterine cervix, provide changes in its biomechanical properties as needed. However, further research is needed to clarify the specific mechanisms of collagen involvement in this process.

There is evidence that changes in the structure and packaging of collagen also depend on the composition of glycosaminoglycans (GAGs) in the extracellular matrix of the uterine cervix. The total GAG content in the uterine cervix increases during pregnancy. In addition to this, there have been significant changes in the composition of GAGs in the uterine cervix [36]. GAGs include unsulfated GAGs, hyaluronan, and sulfated GAG chains, which, in combination with proteins, form proteoglycans. Proteoglycans perform various functions in the uterine cervix, one of the most important of which is the binding of growth factors, regulation of the size of collagen fibrils, the distance therebetween, and, as a consequence, the action of protease enzymes [2, 3]. According to a study by G. Westergren-Thorsson et al, numerous proteoglycans, such as versican, decorin, fibromodulin, and others, are present in significant quantities in the uterine cervix, and their composition may change during pregnancy [44]. The function of proteoglycans is regulated by both the protein part of the molecule and the carbohydrate part, namely, the composition, length, and degree of sulfation of the GAG carbohydrate chain, which occurs in the endoplasmic reticulum and the complex at the stage of post-translational modifications. Thus, changes in the structure of GAG chains can regulate the function of proteoglycans in the uterine cervix during its maturation [39, 44]. However, the specific mechanisms underlying these processes remain unexplored.

Other additional components of the extracellular matrix, even though they are present in the uterine cervix in a rather small amount, also somewhat affect its biomechanical properties. Elastic fibers, which give the uterine cervix the property of distensibility, account for an average of 0.9–2.4% of the total volume of the uterine cervix connective tissue [29, 30]. Although the content of elastic fibers does not change during pregnancy, they may also play an important role in cervical maturation. Thus, it was shown that in women with mutations in genes encoding fibrillin proteins and components of elastin microfibrils, the content of elastic fibers and cervical elongation decrease [5].

Matrix proteins such as secreted cysteine-rich acidic protein (SPARC, osteonectin), thrombospondin 1, thrombospondin 2, and tenascin C are also some of the most important structural components of the connective tissue extracellular matrix. These proteins play important roles in regulating the interaction of cells with the extracellular matrix, wound healing, and cell migration within the connective tissue of many organs of the human body [34]. Given these functions of matrix proteins, they have attracted the attention of researchers studying the morphological and functional aspects of cervical maturation. For example, several studies have found a change in the expression of genes encoding thrombospondins 1 and 2, tenascin C during maturation, and postpartum repair of the uterine cervix in experimental animals and humans [19, 42, 46]. In experimental studies on pregnant laboratory mice with a deficiency of each of these proteins, numerous disturbances in the production and interaction of components of the uterine cervix extracellular matrix were noted, which led to a change in its biomechanical properties [7, 8, 27, 28]. For example, in mice with thrombospondin 2 deficiency, premature softening of the uterine cervix occurred, and the risk of developing premature birth increased [27]. Thus, a change in the expression of these matrix proteins has a significant effect on the process of cervical maturation.

## CONCLUSION

Thus, the process of cervical maturation is complex and affects many structural components of the uterine cervix: epithelial, immune-inflammatory, extracellular matrix elements. With the maturation of the uterine cervix, epithelial cells actively proliferate and differentiate, they significantly increase the expression of some types of protein molecules (connexins, desmogleins, claudins, and several others) aimed at increasing the barrier properties of the mucous membrane. Besides, cervical epithelial cells play an important role in providing protective reactions of innate and acquired immunity by increasing the formation of cytokines, chemokines, and adapter protein MyD88, which can serve as therapeutic targets for the development of pharmaceutical preparations. Immune and inflammatory components of the uterine cervix regulate the structure and properties of the extracellular matrix. Increased activity of pro-inflammatory factors leads to disorganization of the extracellular matrix and contributes to premature birth. Infectious agents, predominantly gram-negative bacteria, are triggers for the early maturation of the uterine cervix. Moreover, during the maturation of the uterine cervix, profound structural changes occur in its extracellular matrix, independently from the factors indicated above. The expression of different types of collagen changes, in particular, type 1 collagen is replaced by type 3 collagen. The composition and properties of glycosaminoglycans, the expression of genes encoding matrix proteins (thrombospondin 1, thrombospondin 2, tenascin C and osteonectin) also change. There are certain conflicting data on the role of individual

components in cervical maturation, and there is also insufficient knowledge of specific mechanisms. Further study of the role of individual components in cervical maturation and clarification of the underlying mechanisms will be critical for the development of diagnostics as well as treatment and prophylactic strategies to handle violations of the process of cervical maturation.

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# FUNDAMENTAL PRINCIPLES AND TECHNIQUES OF EXPERIMENTAL MODELING OF HYPOTHYROIDISM: A LITERATURE REVIEW

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**ABSTRACT** — The purpose of our paper is to discuss principles and techniques of experimental modeling of hypothyroidism in laboratory animals, as well as reviewing advantages and disadvantages of experimental models.
Materials and methods. Comparative analysis of contemporary international biological and medical publications in *PubMed/MEDLINE* and *Embase* databases; analysis of contemporary national scientific sources using *Google Scholar* database.
Results. To date, there are six basic principles of experimental modeling of hypothyroidism, i.e.: dietary, drug, surgical, immunological, radioisotope, genetic. Each of the techniques can be used for simulation of the main conditions for hypothyroidism development. Dietary modeling stands for the iodine intake restriction because iodine is an indispensable component in thyroid hormones synthesis. Drug modeling means the use of antithyroid drugs that block thyroid hormones synthesis. Surgical modeling principle involves thyroidectomy. The principle of immunological modeling of hypothyroidism consists in administration of immunosuppressants to animal body. The principle of radioisotope modeling of hypothyroidism lies in acting with a radioactive isotope of iodine on animal body. Genetic modeling principle leads to stimulation of gene mutations in laboratory animals to encode the thyroid hormones formation or their receptors, and results in appearance of the transcription factors responsible for development of the thyroid gland.
Conclusion. Hypothyroidism is a very common pathological condition affecting many organs and tissues. Thus, employment of the hypothyroidism experimental models to study fundamental pathophysiological and pathomorphological processes represents a scientific and research topic of immediate interest. Each of the hypothyroidism modeling principles is specific, and provides for simulation of particular conditions needed for hypothyroidism development in laboratory animals. Taking into consideration numerous beneficial effects of thyroid hormones upon almost all organs and tissues of human body, it is noteworthy that experimental models of hypothyroidism shall be highly sought after by researchers practicing in all medical specialties.

**KEYWORDS** — experimental simulation technique, hypothyroidism, iodine deficiency, antithyroid drugs, mercazolilum, thyroidectomy, methotrexate, radioactive iodine mutations.

## INTRODUCTION

Hypothyroidism represents a very common clinical disorder arising from deficiency of the thyroid hormones, namely the deficiency of triiodothyronine (T3) and tetraiodothyronine (T4). Average occurrence of hypothyroidism in the developed countries is 4–5%, and still in some countries, the hypothyroidism cases occur much more often. For instance, in India, the occurrence of hypothyroidism reaches around 11% [2, 3]. Hypothyroidism has a gender response (it is more common in women), and is age-related (the prevalence of hypothyroidism is higher among older people) [2].

Functional activity of the thyroid gland is inextricably related to the activity of hypothalamus and pituitary gland. In this regard, the organs group into the hypothalamic-pituitary-thyroid system. With involvement of thyrooliberin and thyroid hormones, the hypothalamus and hypophysis activate formation of the thyroid hormone in follicular cells of the thyroid gland. If the level of thyroid hormones in blood is insufficient, the negative mechanism activates adenocytes in anterior pituitary gland. This results in an increase in thyroid hormone (TSH) levels. Its concentration represents one of the most prominent criteria to diagnose hypothyroidism. In addition, TSH levels often increase during the subclinical hypothyroidism, when the clinical manifestations are yet weak or absent and thyroid hormone levels are still within the reference range [35, 40].

Classification of hypothyroidism depends on the hypothalamic-pituitary-thyroid system level at which the disorder occurs. In primary hypothyroidism, the functional activity of the thyroid gland itself is affected and/or impaired. In secondary hypothyroidism, the hypophysis is initially affected, and this leads to the decrease in TSH formation with subsequent inhibition of thyroid function. In tertiary hypothyroidism,

hypothalamus is affected, and this causes inhibition of pituitary adenocytes and follicular thyroid cells.

Besides, hypothyroidism, in relation to the time of its occurrence (development), can be either congenital or acquired. The most common type of hypothyroidism is primary acquired. Its presentation is explained by the insufficient entry of the trace substance of iodine into human body. Hypothyroidism caused by this is particularly representative of the areas endemic for iodine deficiency. In iodine-abundant areas, the main cause of primary acquired hypothyroidism is Hashimoto's disease (autoimmune thyroiditis) [10, 31]. Post-operative and radiation-induced hypothyroidism also act as remarkable prerequisites for primary acquired hypothyroidism [10, 38]. Congenital hypothyroidism is often explained by either the impairment in thyroid development (thyroid dysgenesia), or the impaired thyroid hormone formation (dyshormonogenesis). The root of these disorders lies in gene mutations encoding the formation of transcription factors, receptors and enzymes [8, 46].

Hypothyroidism in its clinical and morphological expressions is quite variable. This is supported by the fact that thyroid hormones target almost all organs and tissues in human body. The manifestation rate of clinical-morphological disorders depends on the thyroid hormone deficiency severity. According to the range of experimental and clinical studies, hypothyroidism produces quite a great effect on skin and its derivatives [1, 43], bone tissue [15, 19], liver [39], as well as the nervous [36, 41] and cardiovascular systems [13, 17].

For the purposes of studying clinical and morphological expressions of hypothyroidism and performing the pre-clinical evaluation of therapeutic and preventive interventions efficiency, the hypothyroidism experimental simulation techniques are often employed. The general scientific research studies, discussing in detail the fundamental principles and methods used to model hypothyroidism, are not available.

*The purpose* of this research paper is to discuss principles and techniques of experimental modeling of hypothyroidism, as well as reviewing advantages and disadvantages of the existing techniques of hypothyroidism modeling.

## BASIC PRINCIPLES OF EXPERIMENTAL MODELING OF HYPOTHYROIDISM

In the best-case scenario, hypothyroidism modeling techniques shall simulate the main conditions for hypothyroidism development. Taking into account the most frequent causes of hypothyroidism development of in a regular practice, it is possible to highlight the following basic principles of hypothyroidism modeling: maintaining the iodine deficient food routine for laboratory animals (dietary model) [30, 45]; thyroidectomy [21, 43], or coagulation of the arteries feeding the thyroid gland without thyroidectomy (surgical model) [24]; oral administration of antithyroid drugs to laboratory animal body (drug model) [6, 20, 25]; stimulation of particular gene mutations responsible for development of the thyroid gland, and biosynthesis of thyroid hormones (genetic model) [22, 23, 32]; radioactive iodine action to laboratory animal (radioisotope model) [37, 44, 47]; and, finally, administration of the immunosuppressive-active drug (immunological model) [26].

Hereafter, this research paper discusses in detail each of these hypothyroidism modeling principles.

## DIETARY PRINCIPLE OF HYPOTHYROIDISM MODELING

Despite its little amount in bodies of mammals and humans, trace substance of iodine plays an important role in biosynthesis of T3 and T4. During biosynthesis of the thyroid hormone, molecules of iodine connect with amino acid tyrosine being one of the thyroglobulin protein constituents. In order to form thyroid hormones in sufficient quantity, the daily intake of iodine should average in the range of 100–200 μg. Even with minor reductions in iodine intake (up to 40–80 μg per day), the iodine deficiency condition develops, which, if not leading to clinically significant disorders of thyroid function, causes deviation in the central nervous system development [14, 30]. Besides, subclinical iodine deficiency in food results in the higher risk of atherosclerosis and cardiovascular diseases [16].

Hypothyroidism modeling through the dietary models represent more accurately the true-to-life clinical conditions, as the lack of iodine in food becomes the key factor of primary acquired hypothyroidism development. Various experimental research studies discuss experimental simulations carried out in pregnant rats, and are based on the iodine deficient diet. In laboratory group of animals, the decrease in T3 and T4 biosynthesis and the increase in TSH level of both parents and their newborns is observed. In addition, the cognitive impairment is detected in newborns in laboratory group of animals, as well as the drop in motor activity [45]. M. Kulimbetov et al. proposed the iodine deficient diet based on local produce from the regions of Uzbekistan endemic for iodine deficiency. When modeling hypothyroidism using this diet in rats, the decrease in T4 secretion and structural

rearrangement of the thyroid gland was revealed. This consists in the small-follicular adenomas formation, as well as in the gland weight growth [30].

Primary advantages of the dietary principle of hypothyroidism modeling are gradual decrease in iodine levels and proximity to the true-to-life conditions of clinical practice in view of hypothyroidism development. In addition, the advantages imply that complex invasive and surgical methods are not required.

Among the disadvantages are difficulties in designing specific iodine deficient diets, and the need for constant control and accurate calculation of the iodine concentrations sufficient for hypothyroidism development.

## SURGICAL PRINCIPLE OF HYPOTHYROIDISM MODELING

The majority of hypothyroidism surgical models imply complete removal of the thyroid gland (thyroidectomy) in laboratory animals. Thyroidectomy leads to the rapid and persistent drop in thyroid hormone levels [21, 43]. The surgical model enables reproduction of the post-operative hypothyroidism formation which is often observed in clinical practice. Opposite to the dietary model, the surgical simulation of hypothyroidism result in the drop of T3 and T4 concentration much faster. Most often the modeling objects include rats, mice, rabbits, sheep, and dogs.

The research fields involving the surgical models of hypothyroidism vary greatly. For instance, M. Tsujio et al., by means of the surgical models, studied pathomorphological changes in skin, including epidermis and hair follicles, under hypothyroidism. In all rats with thyroidectomy, the slow-down in hair growth was observed 12 weeks after the surgery. Dry and pale skin was also noted, which is representative of hypothyroid condition in humans [43].

M. Helal et al. studied morphological changes in parotid salivary glands in rats under thyroidectomy [21]. In other studies, K. Chen et al. researched in changes in renin-angiotensin-aldosterone system functioning under experimental post-operative hypothyroidism [11, 12]. It is noteworthy, that under hypothyroidism and drop in T3 and T4 levels, the decrease in expression of RNA matrix of the renin enzyme and RAAS inhibition is observed. These changes are associated with the impaired functioning of cardiovascular system, i.e. lowering of heart rate and blood pressure, drop in cardiac output, relaxation of smooth muscle vascular cells, and decrease in total peripheral vascular resistance.

A. Kade et al. proposed another principle of hypothyroidism surgical modeling. It focuses on coagulation of the upper and lower thyroid arteries, which results in thyroid ischemia and drop in T4 and T3 formation. If this model is used, there is no need to dissect the thyroid gland [24].

Among primary advantages of the surgical principle of hypothyroidism modeling are achievement of rapid and persistent hypothyroidism, simulation of conditions needed to develop post-operative hypothyroidism as it is observed in clinical practice.

The disadvantages include the need to involve highly qualified surgical staff to perform operative procedures, complexity of the thyroid glands extraction leading to their thyroidectomy, as well as the decrease in parathyroid hormone formation and drop in calcium levels in blood. Besides, given partial thyroidectomy, development of autoimmune reaction against the remaining part of the thyroid gland is possible. With complete thyroidectomy, parafollicular cells producing calcitonin hormone cease to exist.

## DRUG PRINCIPLE OF HYPOTHYROIDISM MODELING

The drug model of hypothyroidism consists in on oral administration to laboratory animals of antithyroid drugs blocking biosynthesis of thyroid hormones [6, 7, 20, 25, 28, 29]. In accordance with the mode of administration and type of antithyroid drug, there exist some varieties of hypothyroidism drug models. Typical antithyroid drugs used in modeling hypothyroidism are mercazolilum (thiamazole, methimazole, tapazole) and propylthiouracil. Their mechanism of action is based on inhibition of thyroid peroxidase enzyme leading to the decrease in active form of iodine formation and the decrease in thyroglobulin iodization. Antithyroid drugs administration is managed through placement of a special gastric tube, or with required dose of the drug dissolved in drinking water [7, 28, 29]. Differences in drug dosing sufficient to achieve hypothyroidism are defined by interspecific differences. For instance, to induce hypothyroidism in rats, appropriate dosing of thiamazole is 2.5 mg per 100 g of animal weight per day for 21 days [25]. To develop hypothyroidism in rabbits, thiamazole dosing of 2 mg per 1 kg of animal weight per day is administered for 21 days [28]. Studies by F. Kamilov proved that the departure from appropriate dosing (2.5 mg/100 g) towards the decrease (1 mg/100 g) did not cause hypothyroidism development in rats, whilst the departure towards the increase (5 mg or more per 100 g of weight) caused multi-organ pathology abnormal for clinical course of hypothyroidism [25].

In another study by Y. Kruk et al., hypothyroidism in rats was simulated by intragastric administration of mercazolilum dosing 10 mg/kg for 2 to 8 weeks. In that case, after 2 weeks, animals developed

mild hypothyroidism; after 4 weeks, its degree was average; and after 8 weeks, it became severe. Along with progressing hypothyroidism, oxidative stress (lipid peroxidation processes) increased in blood and brain tissues in laboratory rats [29].

Since the method of continuous use of the gastric tube causes various types of technical inconvenience for researchers and produces harmful effect on animal body, it was proposed to use hypothyroidism modeling techniques by administering antithyroid drugs with drinking water. The core benefit of this method over intragastric administration is that the drug is continuously administered causing no stress in animals. Thus, the study by H. Bhargava et al. proposed hypothyroidism modeling for 32 days using the drinking water method and containing 0.05% of methimazole. Clinical symptoms (decreased body temperature, decreased systolic blood pressure and heart rate) as well as laboratory data (decreased thyroid hormone levels and increased TSH levels) pointed to hypothyroidism development. In addition, in test group rats, the rate of body weight gain was significantly lower than in control group rats. This attests to important effect of thyroid hormones on metabolic processes [7].

Primary advantages of the drug principle of hypothyroidism modeling are relative simplicity of modeling without any need for qualified surgical staff; broad accessibility and low cost of antithyroid drugs; good solubility of drugs in water. In addition, advantages imply insignificant resources when modeling hypothyroidism in small laboratory animals (rats, rabbits).

The disadvantages of the drug principle of hypothyroidism modeling represent the need to define accurate drug dose necessary for hypothyroidism development. Technical errors are possible; and there is a need for more consistent monitoring of T3, T4 and TSH concentration in blood serum. In addition, placement of gastric tube causes stress to laboratory animals.

## IMMUNOLOGICAL PRINCIPLE OF HYPOTHYROIDISM MODELING

In view of the fact that endocrine and immune systems are closely related, action upon the latter can significantly affect thyroid function. Experimental study by S. Kashchenko assessed morphofunctional state of the thyroid gland in laboratory rats under conditions of immunosuppressant (methotrexate) and immunomodulators (immunophan) administration. The authors found out that under intramuscular administration of methotrexate at 50 μg, the evident morphological changes in the thyroid gland occur on the 7$^{th}$ day of the experiment, i.e., deformation of follicles, change in follicular thyrocytes cubic form representative of normothyroidism condition into low-prismatic and flattened being the distinctive evidence of hypothyroidism. In addition, it was revealed that the colloid inside follicles was unevenly distributed, and changed its consistency becoming lumpy and stratiform. Rats in test group also manifested the thyroid weight loss by about 10% on the 7$^{th}$ day if compared to control group. In response to immunomodulators administration, thyroid morphology recovery was observed [26]. Consequently, immune system inhibition goes in parallel with thyroid function inhibition.

Experimental study data are consistent with clinical results. Thus, the scientific sources describe cases of hypothyroidism development in the context of cytostatic methotrexate administration [9]. Therefore, immunological principle of simulating hypothyroidism may be of great interest as regards the enhancement in therapeutic and preventive measures for their subsequent introduction into clinical practice.

Primary advantages of the immunological principle of hypothyroidism modeling are relative simplicity of modeling, intramuscularly drug administration and arrival at high bioavailability.

The disadvantages comprise insufficient research into this model, as well as various side effects triggered by cytostatic drugs.

## RADIOISOTOPE PRINCIPLE OF HYPOTHYROIDISM MODELING

The principle of hypothyroidism radioisotope modeling means administration of the radioactive isotope iodine 131I in laboratory animals, which is used in clinical practice to treat hyperthyroidism. Hypothyroidism modeling using radioisotope exposure was mostly carried out in rats and mice. Optimal dosing for animals is 150 μCi that approximately corresponds to the absorbed dose of 0.5 Gy received by the population of the CIS countries at the time of the Chernobyl disaster [44].

V. Usenko et al. completed hypothyroidism simulations in pregnant rats using iodine radioisotopes. At the outcome of simulations, evident changes were found, and were representative of acquired hypothyroidism in both female parents and congenital hypothyroidism in babies of these female rats. T4 level decreased on average by 43%, and through the negative mechanism there was around 8-fold increase in TSH level. The effect of hypothyroidism in female parents on the thyroid gland and nervous system development in fetus depended on the time of exposure to iodine radioisotope. In overall, the newborn rats proved the decrease in body, brain and the thyroid gland weight [44].

C. Reilly et al., simulating hypothyroidism by means of various doses of radioisotope, found that dosing of 50 μCi did not affect much the morpho-functional state of the thyroid gland, whilst dosing of 150 and 450 μCi led to significant drop in thyroid hormone levels and rise in TSH [37]. Radioactive iodine isotope can damage follicular cells that produce calcitonin needed for calcium ion metabolism [5, 42]. Thus, according to the results of experimental studies, after exposure to iodine radioisotope, the number of parafollicular cells in newborns decreases [42], and the number of cells does not recover even 40 days later after exposure to the radioisotope [18]. Taking into consideration this adverse effect of radioisotope on parafollicular cells, it is required to control calcium levels in blood serum.

Primary advantages of the radioisotope principle of hypothyroidism modeling are the achievement of persistent and prolonged hypothyroidism even when employing relatively small amount of iodine radioisotope.

The disadvantages of the radioisotope principle of hypothyroidism modeling represent the need for particular skills to handle radioactive isotopes, as well as negative effects produced by radioisotopes on parafollicular cells.

## GENETIC PRINCIPLE OF HYPOTHYROIDISM MODELING

The genetic principle of hypothyroidism modeling consists in stimulation of specific mutations in genes that encode receptors, transcription factors, and enzymes for thyroid hormone biosynthesis. According to the scientific sources, in about 5% of cases hypothyroidism is caused by gene mutations encoding TSH receptor or the following transcription factors: TITF1, FOXE1 or PAX8.

The research group led by E. Amendola [4] designed the genetic model of hypothyroidism. The basic principle of this modeling is to crossbreed heterozygous mice with specific mutations in genes encoding TITF1 and PAX8 factors to produce double heterozygotes. At that, the combination of two heterozygous zero mutations as per TITF1 and PAX8 causes severe hypothyroidism with significant increase in TSH, sharp decrease in thyroid hormone concentration, body weight loss, thyroid hypoplasia, and a higher risk of thyroid hemiagenesis [4]. It was proved, that development of hypothyroidism when using this model stems from the disruption of thyroid organogenesis due to TITF1 and PAX8 deficiency [27, 34].

K. Johnson et al. studied the hypothyroidism specifics in mice with mutations in genes encoding double oxidase enzyme DUOX2 [23] and thyroid peroxidase [22]. Double oxidase is required to form hydrogen peroxide for thyrooperoxidase enzyme, which further catalyzes iodine conversion to its atomic form for inclusion in thyroglobulin protein. Principal clinical and morphological evidence in these mice were pituitary dysplasia, drop in thyroid hormone levels and increase in TSH concentration. In addition, very particular morphological changes associated with hearing impairment in laboratory animals, were impaired formation of internal furrow and cortical tunnel, as well as thickening of tectorial membrane [23].

Snell dwarf mice with mutations in genes encoding pituitary transcription formation of factor Pit1 represent another model of secondary hypothyroidism. This factor is crucial in view of adenocytes development in anterior pituitary lobe, including thyreotropocytes that synthesize TSH [33].

Among primary advantages of the genetic principle of hypothyroidism modeling is the possibility of congenital hypothyroidism mechanisms in-depth studies. Nonetheless, it will not be possible to reconstruct such conditions using other principles of hypothyroidism modeling. This, in fact, makes genetic models unique.

The disadvantages of genetic principle of hypothyroidism modeling imply the use of expensive and not easily accessible equipment needed for the purposes of molecular genetic research.

## CONCLUSION

Basing on comparative analysis of the relevant sources, we have identified six basic principles of experimental modeling of hypothyroidism, i.e.: dietary, drug, surgical, immunological, radioisotope, genetic. Each of these principles is specific in its own way, and allows reconstruction of initial hypothyroidism conditions, as well as studying its clinical and morphological expressions. In view of the vast prevalence of hypothyroidism and profound effects of thyroid hormones upon nearly all cells and tissues in mammals, the employment of experimental hypothyroidism modeling can be relevant to doctors from different specialties.

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# BASIC PRINCIPLES AND METHODS OF MODELING HYPOGONADISM: A LITERATURE REVIEW

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**ABSTRACT** — Purpose: To consider the basic principles and methods of experimental modeling of hypogonadism in laboratory animals, to define the main benefits and drawbacks of each separate method in hypogonadism modeling.
Materials and methods: We analyzed modern foreign and domestic literature using the following databases: PubMed / Medline, Embase, Google Scholar.
Results: Presently, there are three main principles of modeling hypogonadism: surgical, genetic, and pharmacological. The principle of surgical modeling of hypogonadism is based on the removal of the gonads, or on the temporary imposition of a suture on the distal section of the spermatic cord, which leads to occlusion of the testicular artery that feeds the gonads. The principle of genetic modeling of hypogonadism is to induce mutations in the genes encoding the most important regulatory molecules, in particular kisspeptin, neurokinin B, and their receptors in laboratory animals. The principle of pharmacological modeling of hypogonadism is based on the administration of streptozocin to laboratory animals, which has a toxic effect on the gonads and pancreas.
Conclusion: Hypogonadism represents a very common pathological condition that affects many organs and tissues. Therefore, the use of experimental models of hypogonadism to study fundamental pathophysiological and pathomorphological processes is a relevant research area. Each principle of hypogonadism modeling is unique in its own way, exhibits advantages and disadvantages, and allows the creation of specific conditions necessary for the development of hypogonadism in laboratory animals. Taking into account the numerous beneficial effects of testosterone on many cells and tissues of the human body, it becomes obvious that experimental models of hypogonadism can be in demand for many medical spheres.

## INTRODUCTION

Hypogonadism is a clinical and laboratory syndrome that develops as a result of a decrease in the production of the hormone testosterone by the sexual glands of men [5, 21, 31]. Biosynthesis of testosterone by Leydig's cells is controlled by the hypothalamus and pituitary gland, forming the gonadal-pituitary hypothalamic system: gonadotropin-releasing hormone → luteinizing hormone → testosterone. In hypogonadism, the formation of the gonadotropin-releasing hormone and the luteinizing hormone is enhanced by the negative regulatory feedback mechanism [5, 21].

Hypogonadism is classified into several types depending on the level at which the disorder takes place. In primary hypogonadism, testosterone production in the male gonads is disturbed, most often affected by inflammatory or tumor processes, removal, and decrease in activity with age. Primary hypogonadism is also called hypergonadotropic hypogonadism, since low androgen levels through long and short negative regulatory feedback mechanisms stimulate the synthesis of gonadotropin-releasing hormone and luteinizing hormone by the hypothalamus and pituitary gland, respectively. Whereas secondary hypogonadism is caused by the insufficient formation of gonadotropin-releasing hormone and luteinizing hormone as a result of damage to the hypothalamus and pituitary gland, and consequently, insufficient stimulation of the gonads to produce androgens [5, 21, 31].

The clinical picture of hypogonadism depends on the age at which the disease occurs, as well as the form and degree of testosterone deficiency. The testosterone molecule in the human body performs many important functions, so low testosterone levels lead to numerous disorders. To a greater extent, hypogonadism affects the musculoskeletal system, reproductive, nervous, and cardiovascular systems [5, 8, 11, 16–18, 21, 31, 35, 38]. Methods of experimental modeling of hypogonadism in animals are widely used to study the structural and functional disorders of various organs and systems that arise during hypogonadism. Review articles that fully summarize the basic principles and methods of experimental modeling of hypogonadism are not available.

*The purpose* of this review is to discuss the principles and methods of experimental modeling of hypogonadism, to analyze the advantages and drawbacks of certain modeling methods.

An ideal model of hypogonadism should meet the following criteria: persistent impairment of hormonogenic and reproductive functions, technical availability, and low cost of maintenance, as well as the absence of nonspecific (toxic and adverse) effects on other organs and systems when modeling hypogonadism [19]. Currently, there are three main principles of modeling hypogonadism: surgical, pharmacological, and genetic, however, none of them meets all of the above criteria for an ideal model. Below we will take a look at the principles of modeling hypogonadism.

## THE PRINCIPLE OF SURGICAL MODELING OF HYPOGONADISM

Surgical models of hypogonadism are among the most commonly used and are based on surgical removal of the gonads (unilateral or bilateral orchiectomy/gonadectomy) from male animals [7, 13, 15, 25, 43]. Animals are usually anesthetized with xylazine (10 mg/kg body weight) and ketamine (70 mg/kg body weight). The surgical access is achieved through the midsection of the scrotum about 1 cm long. During the operation, the testicles are removed after the spermatic cords are crossed [13]. The disadvantage of this model is the complexity of implementation and the need for qualified surgical personnel. To confirm the simulated conditions, the levels of hormones that make up the gonadal-pituitary hypothalamic system (namely, testosterone, luteinizing hormone) are assessed; evaluation of the mass and size of the gonads, and reproduction capability test are implemented [19].

Native researchers P. Kulikova et al. developed another surgical model of hypogonadism [19]. The principle of this model is based on the temporary ligation of the distal section of the spermatic cord, which leads to partial occlusion of the testicular artery. To establish the optimal time for clamping the spermatic cord, the researchers applied a suture using a surgical needle and thread for a period of one, two, three, and four days (four experimental groups of animals). Serum testosterone concentration was determined using an automatic enzyme-linked immunosorbent assay. A month after the temporary ligation of the spermatic cord, all groups of animals showed a decrease in the weight and linear dimensions of the gonads by 1.5–7 times in comparison with the control group ($p < 0.05$). The minimum time required for the development of persistent hypogonadism, according to the study, comprised 3 days. The testosterone level in these animals was lower than that in the group of intact males, however, the degree of decrease was not significant, which, most likely, was because the suture was applied to only one spermatic cord. The reproduction capability test showed negative results. Ligation for more than 3 days can be dangerous due to the development of pronounced necrotic changes in the testes [19].

## THE PRINCIPLE OF GENETIC MODELING OF HYPOGONADISM

As noted earlier, the production of testosterone by the gonads is inextricably linked to the optimal activity of the hypothalamus and pituitary gland. Deficiency of gonadotropin-releasing hormone or luteinizing hormone leads to decreased testosterone production and is termed hypogonadotropic hypogonadism. Congenital or idiopathic hypogonadotropic hypogonadism, the causes, and mechanisms of which have been unknown for a long time, is most often based on genetic disorders. Over the past few decades, genetic studies have been carried out on patients with idiopathic hypogonadotropic hypogonadism, which have revealed several pathways that affect the migration of neurons that produce the gonadotropin-releasing hormone, as well as the secretion and activity of this hormone [3]. In patients with idiopathic hypogonadotropic hypogonadism, mutations were found in the genes encoding kisspeptin (KISS-1), neurokinin B (TAC3), and their respective receptors (GPR54 and TACR3) [10, 14, 39, 40]. The neuropeptides kisspeptin and neurokinin B, in addition to the third peptide hormone, dynorphin, are co-expressed in a specific cell population of hypothalamic neurons and appear to be critical components that regulate the release of gonadotropin-releasing hormone. The expression of genes encoding the formation of these peptides increases during puberty. It was found that the deficiency of kisspeptin and neurokinin B leads to various manifestations of hypogonadotropic hypogonadism, in particular to the absence of puberty and infertility, both in humans and in animals, which indicates the important role of these hormones in the regulation of puberty [20, 44]. Experimental studies have shown that the administration of kisspeptin stimulates the secretion of gonadotropin-releasing hormone and luteinizing hormone [34].

The use of kisspeptin and neurokinin B-deficient mice models allows for a more in-depth study of the mechanisms by which these neuropeptides regulate hypothalamic control of reproduction. KISS-1 gene knockout in mice led to a sharp drop in the level of free testosterone in blood plasma compared to wild-type mice (<0.17 pg/ml versus 4.1 ± 1.8 pg/ml, respectively). Similarly, plasma levels of luteinizing hormone were significantly lower in KISS-1 knockout male mice than in wild-type mice (0.28 ± 0.01 and 0.42 ± 0.03 ng/ml, respectively) [9]. A key advantage of genetic models of hypogonadism is the ability to

study the role of a particular gene and its product (peptide compound) in the development of this disease. Moreover, these models are indispensable for the study of hereditary hypogonadism and preclinical assessment of the effectiveness of medications under development. At the same time, high-tech and expensive equipment are needed to model idiopathic hypogonadotropic hypogonadism, which becomes a drawback of genetic models.

## THE PRINCIPLE OF PHARMACOLOGICAL MODELING OF HYPOGONADISM

The syndrome of hypogonadism develops in several different ICDs: kidney diseases [41], hepatic and thyroid diseases [30], and very often in diabetes mellitus [22, 23, 42]. The mechanisms of the development of hypogonadism in these diseases are different. Due to the high prevalence and steady increase in the incidence of diabetes mellitus [6], and hypogonadism syndrome in diabetes mellitus, as well as the possibilities of using such data in experimental studies, in this article, we will focus on the mechanisms of hypogonadism development in diabetes mellitus.

The prevalence of hypogonadism in patients with diabetes mellitus averages 37–57% [1]. It is reported that approximately 50% of men with diabetes mellitus have reproductive disorders, including hypogonadism, impaired spermatogenesis, psychosexual dysfunction due to depression associated with chronic disease, and erectile dysfunction [42]. According to a study by Al Hayek et al., 36.5% of men with diabetes mellitus have low testosterone levels. Among these patients with diabetes mellitus and low testosterone levels, 16.9% had symptoms of primary hypogonadism, and 83.1% had secondary hypogonadism [1].

Based on clinical observations on the frequent occurrence of hypogonadism in patients with diabetes mellitus, experimental models of hypogonadism have been developed [33, 37]. To simulate diabetes mellitus and hypogonadism, laboratory animals are injected with streptozocin, an alkylating chemotherapeutic medication. It is seldom used in clinical practice due to its high toxicity to the beta cells of the pancreas. In the experimental study of L. Seethalakshmi, after the administration of streptozocin to rats, a pronounced decrease in body weight and size of reproductive organs, a decrease in the number and motility of spermatozoa, and a decrease in the concentration of serum testosterone and luteinizing hormone were noted. While the introduction of insulin or testosterone led to the restoration of the abovementioned indicators [33]. It is assumed that diabetes mellitus can cause hypogonadism through many mechanisms, affecting various links of the gonadal-pituitary hypothalamic system, including suppression of the secretion of gonadotropin-releasing hormone, testosterone, or direct impairment of spermatogenesis. The introduction of insulin and streptozocin significantly affects the functioning of the gonadal-pituitary hypothalamic system. Normally, the gonadotropin-releasing hormone is secreted by the cells of the median elevation of the hypothalamus into the portal circulation by impulses every 1–2 hours, causing the corresponding impulse secretion of luteinizing hormone by pituitary gonadotropic cells. Since the gonadotropin-releasing hormone is not secreted into the peripheral circulation, the assessment of pulsatile luteinizing hormone secretion is the gold standard for measuring gonadotropin-releasing hormone secretion. The study showed that in diabetes mellitus caused by the administration of streptozocin, the pulsatile secretion of LH in mature male rats is significantly reduced [12]. R Steger and colleague comprehensively studied endocrine and sexual function in adult male rats which undergone insulin therapy twice daily after simulating streptozocin-induced diabetes mellitus. With the introduction of streptozocin (50 mg/kg of animal weight), there is a decrease in the level of testosterone in the blood plasma of laboratory animals by 4 times compared with the control. The introduction of insulin led to the normalization of the pulsatile secretion of luteinizing hormone and testosterone concentration [36].

It is noteworthy that the restoration of ejaculatory function depends on the time of initiation of insulin therapy. Thus, the ejaculatory function in rats was almost completely restored to normal when insulin therapy was carried out timely, but only partially recovered when insulin therapy was carried out 4 weeks after streptozocin injection [36]. These data suggest that insulin signaling plays a critical role in maintaining the reproductive function of the hypothalamus. And a more prolonged inhibition of sexual function by streptozocin injections can reduce the effectiveness of the subsequent normalizing effect of insulin treatment.

Interesting are the observations of researchers regarding the fact that the pulsatile secretion of luteinizing hormone is significantly lower in castrated rats with streptozocin-induced diabetes mellitus compared to castrated rats without the same. There was also a significant decrease in the sensitivity of the pituitary gland to the exogenous gonadotropin-releasing hormone by about 67% in castrated rats with diabetes mellitus compared to control ($p = 0.001$) [12]. This indicates that hypogonadism caused by diabetes mellitus may lead to dysfunction of the pituitary gonadotropic cells.

In addition to disrupting the secretory function of the hypothalamus and pituitary gland, there

is evidence that diabetes mellitus can directly disrupt the endocrine function of the gonads. The administration of large doses (100–200 mg/kg) of streptozocin to male rats causes a decrease in testosterone production in the male gonads [32]. Streptozocin-induced diabetes mellitus is accompanied by hypogonadism due to a reduced number of functioning Leydig's cells in the gonads and impaired androgen biosynthesis in the remaining Leydig's cells [29].

Insulin is expressed in the gonads and regulates normal Leydig cell function by stimulating deoxyribonucleic acid synthesis and steroidogenesis during puberty. Also, insulin is of great importance for the functioning of Sertoli's cells, since it provides the transport of glucose and the synthesis of lactate, which is an important energy substrate for maintaining the vital functions of germ cells. Therefore, diabetes-related effects on male sex gland function may result from decreased insulin signaling and impaired energy metabolism [24]. In another study, the number and function of Leydig's cells were markedly reduced in rats with streptozocin-induced diabetes mellitus compared to control. Besides, some diabetic rats showed a decrease in insulin-like growth factor-1, androgen receptors after streptozocin injection compared to a control group of animals [4]. The mechanisms, by which insulin regulates the function of cells of the hypothalamus and gonads, in particular the biosynthesis of hormones, have not been completely established.

Researchers J Orth et al. described morphological ultrastructural changes taking place in Leydig's cells in streptozocin-induced diabetes mellitus [28]. Morphological changes in Leydig's cells caused by diabetes mellitus in adult male rats were not profound and included basically the accumulation of lipid droplets and a decrease in the size (atrophy) of the smooth endoplasmic reticulum. Secondary lysosomes and autophagic vacuoles-like structures were also found in the cytoplasm of Leydig's cells in rats with diabetes mellitus [28].

According to J O'Neill et al., Streptozocin can have a cytotoxic effect on Sertoli's cells, cause oxidative stress and DNA damage [27].

Diabetic models of hypogonadism have an advantage over surgical and genetic models in a way they are relatively easy to perform. As a disadvantage, it should be noted that persistent impairment of reproductive functions and hypogonadism does not occur in all animals, and streptozocin, being a highly toxic compound, causes several additional disorders that can affect the results of the experiment. Although, given the high prevalence of diabetes mellitus accompanied by the syndrome of hypogonadism, experimental diabetic models of hypogonadism are practically indispensable for the study of pathogenetic mechanisms and preclinical assessment of the effectiveness of therapeutic and prophylactic effects. Thus, N Ayuob et al. recently studied the effectiveness of oral antidiabetic medications (metformin, pioglitazone, and sitagliptin) on the sexual function of rodents with streptozocin-induced diabetes mellitus. After treatment with metformin, an increase in testosterone levels and a normalization of the sexual function of animals were observed. The group of mice treated with metformin restored the mass of the gonads to values similar to those in the control group. In contrast, rats receiving other hypoglycemic medications (pioglitazone and sitagliptin) had significantly lower testosterone levels compared with the control group, and there was no restoration of gonads [2]. These data suggest that metformin may be the best antidiabetic treatment option for young patients with diabetes mellitus (especially with a decline in sexual function) compared with pioglitazone and sitagliptin.

Low testosterone levels were also observed in the group of diabetic mice deprived of oral hypoglycemic agents. In the gonads and epididymis of male diabetic rats, unfavorable histopathological changes caused by lipid peroxidation and DNA damage due to the accumulation of reactive oxygen species were found [2]. In this regard, the study of the role of oxidative stress in the pathophysiology and pathomorphology of hypogonadism in diabetes mellitus is a very interesting direction in terms of developing supplementary therapy.

Exercise can also be considered as one of the complementary treatments to restore testosterone levels, erectile function, and improve insulin sensitivity in animal models of metabolic syndrome. Rabbits on a high-fat diet develop glucose intolerance and reduction of testosterone levels similar to how humans do. After running on a treadmill specifically designed for use in rabbits, testosterone levels were found to be negatively related to glucose levels and positively related to distance traveled [26].

## CONCLUSION

Thus, for the simulation of hypogonadism in experimental conditions, there are three main principles available to researchers: surgical, genetic, and pharmacological. Each method of experimental modeling has certain benefits and drawbacks that must be taken into account when planning an experimental study. Given the high prevalence of hypogonadism syndrome and the involvement of many organs and tissues in the pathological process, the principles of experimental modeling of hypogonadism can be useful for researchers of various medical specialties and may enable

studying the necessary aspects of its manifestations and conducting a preclinical assessment of the effectiveness of the developed medications.

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# STUDY OF HEMOMICROCIRCULATION IN UPPER-EXTREMITY SKIN IN HEALTHY MEN IN NORMAL CONDITIONS WITH ACCOUNT OF HANDEDNESS

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**ABSTRACT** — In this study we used laser doppler flowmetry to investigate the parameters of peripheral blood flow in the upper extremities in young males both right- and left-handers. Based on the data obtained we found that in right-handers (dextrals) active mechanisms of regulation of blood microcirculation prevail on the leading hand, In left-handers (sinestrals) active and passive mechanisms of its regulation are involved in the regulation of blood flow on the leading hand (left) and on the opposite (right). However, the contribution of active mechanisms is lower than that of right-handers.

**KEYWORDS** — hemomicrocirculation, laser doppler flowmetry, asymmetry.

## INTRODUCTION

Prevention of circulatory disorders requires creation of a database containing normal values of hemomicrocirculation indicators. The relevance of the concept of *asymmetry* was realized at the end of the XX century due to its significance in science in general and in biology in particular. It is known that when viewed in detail, the axial symmetry of the human body is largely conditional — the left half of the face is not similar to the right, the right hand to the left, the left leg to the right. The leading hand is considered an individual sign of psychomotor development, arising from the unequal development of motor skills in the left and right hands [2].

A person who is more right-handed is called a right-handed person (dextral), and someone who uses the left hand more often is called a left — handed person (sinestral). A minority of people are equally skilled with both hands — they are called Ambidextrous. According to the literature, approximately 8–15% of the adult population were left-handed [7]. Studies indicate that men are more likely to be left-handed than women [8]. Left-handers are most common among identical (monozygotic) twins [3], and some groups of people with neurological disorders such as epilepsy [7], autism [1], mental retardation [4] and dyslexia [6]. Laser doppler flowmetry (LDF) is a modern non-invasive method for evaluating the microcirculatory system. For diagnostics, the tissue is probed by laser radiation; processing reflected from the tissue radiation based on allocation of registered signal Doppler frequency shift of the reflected signal proportional to the speed of motion of particles in the microvasculature; the ongoing research is the registration of changes of blood flow or lymph flow in the microvasculature — flowmetry [5].

In this regard, the study of the parameters of local tissue blood flow in the norm, with account of the predominant hand, is relevant in terms of diagnosing circulatory disorders and choosing an adequate treatment method.

*The aim of the study* was to simultaneously study hemomicrocirculatory parameters in the upper-extremity skin of healthy men in normal conditions.

## MATERIALS AND METHODS OF RESEARCH

Twenty-three young men (medical students) aged 19 to 23 years were examined in the laboratory of the Department of Human Anatomy (RUDN University, Moscow, Russia). Informed consent for participation in the study was obtained from all subjects. Hemocirculation was studied using laser Doppler flowmetry (LDF) in the skin of the third finger of the left and right hand. When analyzing the results of the study, the sign of the predominant hand (sinestrals and dextrals) was taken into account, the subjects were in the supine position. The study of blood flow was performed using a new approach of a distributed system with two laser analyzers "LAZMA PF". The laser analysers were designed by LLC NPF "LAZMA", Russia» for individual assessment of micro-blood flow (with a fixed fiber-optic probe). Analyzing dopplerograms we

determined the average value of the microcirculation index (perfusion index) — (M) in perfusion units (PF. units), the average square deviation of the amplitude of blood flow fluctuations from the arithmetic mean value — (σ), the coefficient of variation — (KV). The last two parameters reflect blood flow modulation. The tissue perfusion index determined by the LDF method is proportional to the product of the number of red blood cells and the average speed of their movement (range 0.5–5 mm/s). The total time of the recording of hemomicrocirculation was 5–6 minutes. The rhythmic structure of oscillation was studied using LDF-gram spectral analysis: active (endothelial — VLF, neurogenic — NF and myogenic — MF) and passive modulations (cardiac — CF, respiratory — RF). The hemocirculatory parameters (PM) recorded in LDF, the amplitude of active and passive blood flow modulations, were processed using variational statistics methods, the significance of differences was determined using the student's criterion, and the results were considered reliable at $p < 0.05$

## THE RESULTS OF THE STUDY

The index of microcirculation (PM) on the upper limbs in the studied groups of left- and right-handers ranged from 18 to 21 in perf. units and the average was 19.5 ± 0.32 mm perf. units.

The results of the study of the upper limb in sinestrals showed that the microcirculation index is higher in the left limb than the right limb (Table 1). The microcirculation index in the right upper limb in dextrals is higher than the values in the left limbs.

The index of blood flow modulation (σ) on the upper right limb in sinestrals is slightly higher than on the left limb, which may indicate some differences in the mechanisms of blood flow modulation. In dextrals it did not change statistically significantly, which indicates that the mechanisms of blood flow modulation are preserved (Table 1).

The coefficient of variation (Kv) defines the relationship between perfusion and the magnitude of its variability, so the source it was significantly different from sinestrals and dextrals depending on the dominant hand (Table 1). Differences in this indicator may indicate differences in the conditions of the microcirculation in the left and right hand, a characteristic that dominant hand, values lower than the opposite (Table 1).

MC indicators (PM, mean square deviation of the amplitude of blood flow fluctuations from the arithmetic mean, coefficient of variation) reflect only the general state of the blood MC. A detailed analysis of the functioning of the microcirculatory bed is possible when studying the amplitude-frequency rhythms (modulation) of blood flow with an assessment of certain mechanisms of perfusion control.

The results of the study showed that the skin of the dominant hand (left) in sinestrals among the active modulation is dominated by neurogenic (NF) and the passive modulation of the heart (CF). On the right hand sinestrals have more pronounced respiratory (RF) modulation among the passive ones, and myogenic (MF) oscillation among active ones (Fig. 1).

In dextrals, endothelial modulation (VLF) predominate in the skin areas of the left and right upper extremities, and the indicators are higher on the leading arm (right). On the leading arm of dextrals, the indicators of neurogenic modulation are higher than on the left, and the values of passive blood flow modulations (respiratory and cardiac), on the contrary, are lower compared to the left limb (Fig. 1).

***Table 1.*** *Average indicators of microcirculation in male subjects*

| Indicators | Senestrals | | Dextrals | |
|---|---|---|---|---|
| | Left hand | Right hand | Left hand | Right hand |
| PM, perf. ed. | 21,73+1,092 | 18,25+0,910* | 18,69+0,340* | 19,34+0,318 |
| σ, perf. ed. | 2,74+0,464 | 3,59+0,278* | 2,57+0,516 | 2,76+0,519 |
| Kv, % | 16,20+1,100 | 32,95+3,261* | 29,12+0,791* | 25,53+0,956 |

** P < 0,05 — the differences are significantly different when compared with the data of the leading hand.*

## DISCUSSION AND CONCLUSION

Currently, the LDF method is widely used in various fields of medicine. Obtaining specific data on the state of microcirculation, taking into account individual anatomical and functional features of the body, allows us to take them into account in the diagnosis and treatment of cardiovascular pathology. During the analysis of the results we obtained, it was revealed that the upper limb in men revealed an asymmetry of blood

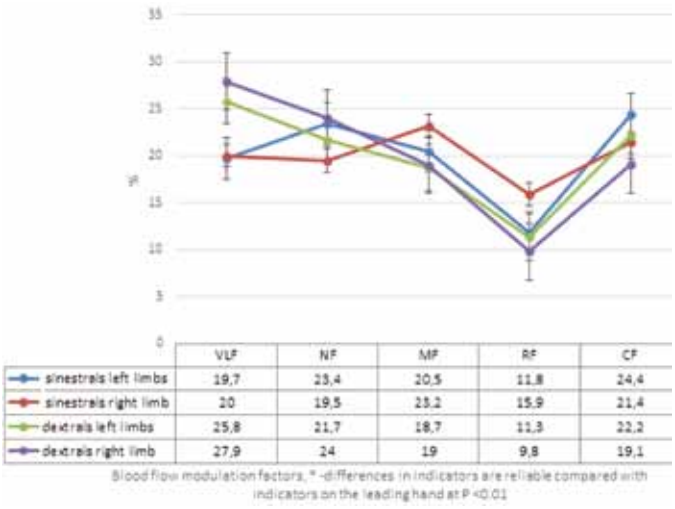


***Fig. 1.*** *Normalized values of the amplitude of active (VLF, NF, MF) and passive (RF, CF) modulations of microcirculation,%,*

circulation associated with the sign of the leading hand — the values of the microcirculation index are higher on the leading hand. Apparently, this is due to a greater functional load on the leading hand in the process of daily life, which leads to an increase in peripheral tissue perfusion.

Based on the analysis of the upper extremity blood flow modulation index, it can be concluded that sinestrals have different modulation mechanisms compared to dextrals. This was confirmed in the study of the spectrum of modulation microcirculation. It was found that under normal conditions, the indicator of tissue hemomicrocirculation in the skin areas of the leading hand (right) extra low primarily affects endothelial and neurogenic modulation, that is, the active modulation of blood flow. On the left hand of dextrals, these indicators are lowered, but at the same time, the values of respiratory and cardiac (passive) modulation are higher. Sinestrals on the leading hand (the left) is dominated by neurogenic (active) and heart (passive) modulation. The right hand of senestrals dominated by myogenic (active), breathing and respiratory modulation (passive).

Thus, the obtained data lead us to conclude that right-handers (dextrals) have active mechanisms of blood circulation regulation on the leading hand, which are less pronounced on the opposite (non-leading) hand. In left-handers (senestrals) on the leading hand (left) and on the opposite (right), active and passive mechanisms of its regulation participate in the regulation of blood flow, but the contribution of active mechanisms is lower than in right-handers.

Thus, the use of laser doppler flowmetry to detect changes in the magnitude and/or ratio of active and passive modulation indicators can serve as a method for diagnosing violations of the mechanisms of microcirculation regulation in various pathological conditions. The data obtained by us can be used both for the diagnosis of blood flow disorders in inflammatory, circulatory, and tumor processes in the upper and lower extremities, and for evaluating the effectiveness of the treatment.

## FUNDING

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# HISTOMORPHOLOGICAL ALTERATIONS IN THE LUNGS IN ACUTE COMBINED BACLOFEN AND ETHANOL POISONING

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**ABSTRACT** — *A generic Baclofen, also known under the brand name of Lioresal*, is a derivative of gamma-aminobutyric acid. Due to its psychotropic effect it is often used as a drug of abuse and for criminal poisoning. An experimental study was carried out on 5 Wistar rats (versus 5 intact rats of the control group) exposed to combined Baclofen (at a dose of 85 mg/kg) and ethanol (7 ml/kg of 40% ethanol) poisoning. We have identified a complex of pathological changes in the lungs of the rats in the early period after the experiment. The outcomes included circulatory disorders of the pulmonary microvasculature (plethora of capillaries, venules), emphysema, atelectasis and dystelectasis, infiltration of white blood cells into intraalveolar septa and thickening of intraalveolar septa due to edema. To quantify the severity of histomorphological changes in the lungs a morphometric study is required.

**KEYWORDS** — Baclofen, ethanol, poisoning, lungs, histomorphological changes.

## BACKGROUND

Baclofen known under the brand name Lioresal is a myorelaxant [1, 2]. This drug is a derivative of gamma-aminobutyric acid and an agonist of GABA (specifically the GABAB ) receptors [3, 4].

Baclofen is available in oral and intrathecal forms [1]. The indications to the drug are as follows: multiple sclerosis, muscular spasticity, some spinal cord diseases, such as tumors, infectious diseases, injuries, acute disorders of cerebral circulation, meningitis [2]. Baclofen has been shown to be effective in the treatment of alcohol addicts [5–10] and patients with cerebral palsy [11].

Adverse effects of Baclofen may include headache, drowsiness, dizziness, weakness, fatigue, nausea and vomiting, urinary retention, constipation [3].

Baclofen has a psychoactive effect and can be a subject to abuse in drug addicts, especially in young people [12]. They often use Baclofen with alcohol drinks. Acute combined Baclofen and ethanol poisonings can be a result of an accidental overdose, criminal actions, or suicidal behavior.

The lung is known to be a target organ in such intoxications. At the same time, the data on morphological changes in such poisoning is limited.

*The objective of the study* was to assess histomorphological changes in the lungs in acute combined Baclofen and ethanol poisoning 3 hours after the administration.

## MATERIAL AND METHODS

Experimental studies were performed on 10 Wistar rats. The animals were divided into 2 groups (the controls and the experimental group). The controls included 5 intact rats. The experimental group included 5 animals treated with Baclofen at a dosage of 85 mg/kg and ethanol (7 ml/kg 40%).

Keeping animals and working with them were carried out in accordance with the European Convention for the protection of vertebrates used for experiments or other scientific purposes (Strasbourg, 18.03.1986).

The lungs were fixed in 10% neutral formalin and immersed into paraffin. Histological sections were processed according to the standard method and stained with hematoxylin and eosin. They were examined by light microscopy using Nikon Eclipse E-400 microscope with a video system based on the Watec 221S camera (Japan) at 400× magnification.

The signs assessed were as follows: emphysema, atelectasis and dystelectasis, thickening of the interalveolar septa due to edema, WBC infiltration of the interalveolar septa, capillary and venous plethora, sludge, hemorrhages in the interalveolar septi and alveoli, the presence of secretion in the lumen of the bronchi.

In order to confirm the reliability of the appearance of a particular histological sign, we used the Fischer ratio. The presence of a histological sign was

considered to be reliable if it did not appear in any cases in one group and appeared in 4 or 5 cases in the other.

## RESULTS AND DISCUSSION

No pathological changes were observed in the lungs of the controls. The alveoli were intact, airy. There were small areas of dystelectasis, located subpleurally. There were no signs of emphysema. Circulatory disorders such as venous, capillary plethora, hemorrhages in the interalveolar septa and alveoli were not observed either. The lumen of the bronchi was free.

In the lungs of the animals treated with the combination of Baclofen (at a dose of 85 mg/kg) and ethanol (7 ml/kg of 40% ethanol) the lumen of the alveoli was free, not expanded. Small areas of emphysema located mainly subpleurally were observed. A large number of areas of distelectasis was found (Fig. 1).

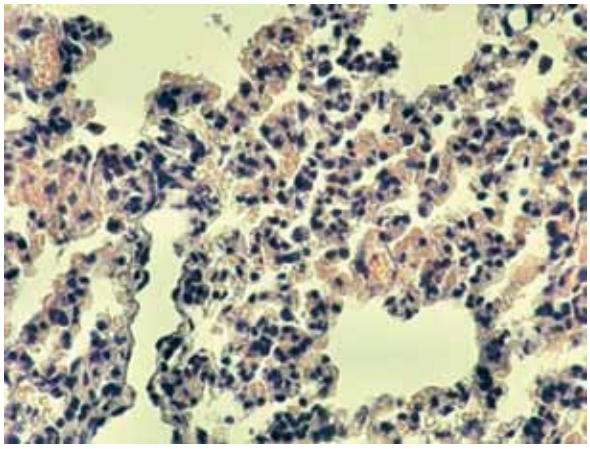

***Fig. 1.*** *Baclofen (85 mg/kg)+ethanol (7 ml/kg 40%). Lung of rat. Distelectasis. Hematoxylin, eosin. Magnification 400*

A large cluster of macrophages was observed in the lumen of the alveoli. No sludge was observed in the pulmonary arteries. All intraalveolar septi were infiltrated with WBCs. There was secretion in the lumen of some bronchioles. Thickening of the interalveolar septi due to edema was observed.

Baclofen is known not to have a direct toxic effect on the bronchi and the lungs. However, this drug increases the presynaptic blockade of nerve impulses generated in the spinal cord, which causes suppression of their transmission. As a result, muscular relaxation, including relaxation of respiratory muscles, occurs. This may lead to difficult breathing and hypoxia. The effects of a GABA receptors stimulation on smooth muscles of the bronchi and on the lungs are also of great importance. Stimulation of GABAA receptors causes contraction of smooth muscles of the bronchi, bronchioles, which is accompanied by spasm and breathing difficulties [13, 14]. Baclofen selectively stimulates GABAB receptors but in high doses it causes GABAA receptor stimulation as well. We observed this effect in the study group. Emphysema was observed in the lungs of the animals. Vascular-tissue permeability increases under hypoxia. According to the literature vascular-tissue permeability increases when GABA receptors are stimulated [15], which is also confirmed by the results of our studies. Thickening of interalveolar partitions due to edema was observed in the experimental group.

## CONCLUSION

As a result of the study we identified a complex of pathological changes in the lungs of the rats in the early period after combined Baclofen and ethanol administration, which included circulatory disorders in all the elements of the microcirculatory bed (plethora of capillaries, venules), emphysema, atelectasis and dystelectasis, WBC infiltration into intraalveolar septa and thickening of intraalveolar septa due to edema. To quantify the severity of histomorphological changes in the lungs a morphometric study is required.

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# CLOZAPINE AND CLOZAPINE-ETHANOL POISONING AS A CAUSE OF HISTOMORPHOLOGICAL CHANGES IN THE CEREBELLUM

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**ABSTRACT** — Clozapine is an atypical neuroleptic with a narrow therapeutic index which is widely used in clinical practice. Due to its psychotropic effect the drug is often misused. Experimental studies were performed on 25 outbreed mail rats. The animals were divided into 5 groups (the controls and four experimental groups). The controls included 5 intact rats. The experimental groups 1 and 2 were treated with clozapine at a dose 150 mg/kg. The experimental groups 3 and 4 received clozapine at the same dose and ethanol (2 ml/kg 70% ethanol). Severe damage to the Purkinje cells of the cerebellum was confirmed by an increase in the number of neurons that register signs of both reversible and irreversible damage. Purkinje cell alteration has increased by 24 hours from the beginning of the experiment.

**KEYWORDS** — clozapine, ethanol, poisoning, cerebellum, histomorphological changes.

## INTRODUCTION

Poisoning is one of the most important issues of forensic medicine and is among the first three main reasons of violent death [1].

Clozapine poisoning is quite common in forensic medical practice. This substance is an atypical neuroleptic. It has a pronounced sedative and antipsychotic effect. Clozapine is widely used in clinical practice to treat acute and chronic forms of schizophrenia, psychosis, manic conditions, bipolar disorders and others. It is considered not to provoke extrapyramidal reactions. Clozapine has been shown almost not to have an effect on the level of prolactin in the blood [2].

According to Russian scientists, about 1 million patients undergo clozapine therapy every year in more than 60 countries of the world [3], which determines the high risk of poisoning with this drug. The therapeutic threshold of clozapine is comparatively narrow. Its single therapeutic dose is 50–200 mg, the highest daily dose is 900 mg, whereas a fatal clozapine dose for an adult is about 2 g [4, 5]. In addition to accidental clozapine poisonings, there is a large number of criminal poisoning with this substance. There is also a large number of combined clozapine-ethanol poisonings [6] .

Clozapine is metabolized in the liver. Its main metabolites are desmethylclozapine (norclozapine) and clozapine-N-oxide. According to the literature, clozapine and its metabolites are excreted by the kidneys [7]. The main target organ in case of such poisonings is the brain.

*The objectives of the study* was to estimate histomorphological changes in the cerebellum in acute clozapine and combined clozapine-ethanol poisonings 3 and 24 hours after the intoxication in the experiments on laboratory rats.

## MATERIAL AND METHODS

Experimental studies were performed on 25 outbreed mail rats. The animals were divided into 5 groups (the controls and four experimental groups). The controls included 5 intact rats. The experimental groups 1 and 2 were treated with clozapine at a dose 150 mg/kg. The experimental groups 3 and 4 were treated with clozapine at the same dose and ethanol (2 ml/kg 70% ethanol). Groups 1 and 3 were euthanized 3 hour after the administration of the drug; groups 2 and 4 were euthanized 24 hours after the administration of the drugs.

Keeping animals and working with them were carried out in accordance with the Directive 2010/63/EU of the European Parliament and of the Council of the European Union on the protection of animals used for scientific purposes.

The samples were fixed in 10% neutral formalin and immersed into paraffin. Histological sections were processed according to the standard method and stained with hematoxylin and eosin and Nissl-staining. The sections were examined by light microscopy using Nikon Eclipse E-400 microscope with a video system based on the Watec 221S camera (Japan) at 400× magnification.

Neuron damage was assessed according to a classification including: 1) acute swelling 2) primary irrita-

tion 3) hydrophic changes 4) fat dystrophy 5) calcification 6) shrinking 7) severe changes 8) ischemic change 9) karyocytolysis 10) *shadow cells* 11) neuronophagy 12) satellite disease 13) pigmented dystrophy [8].

## RESULTS AND DISCUSSION

In the group of controls reversible changes in cerebellar Purkinje cells primary irritation, acute swelling were found (15–20%). The share of irreversible damage to the Purkinje cells of the cerebellum (*shadow cells*, shrinking of neurons, etc.) was about 2–5%.

3 hours after clozapine administration an increase in the number of Purkinje cells with signs of both reversible (60–70%) and irreversible (30–40%) damage was detected.

24 hours after clozapine poisoning the number of Purkinje cells with signs of reversible(40–50%), irreversible (45–55%) damage increases (fig. 1, 2).

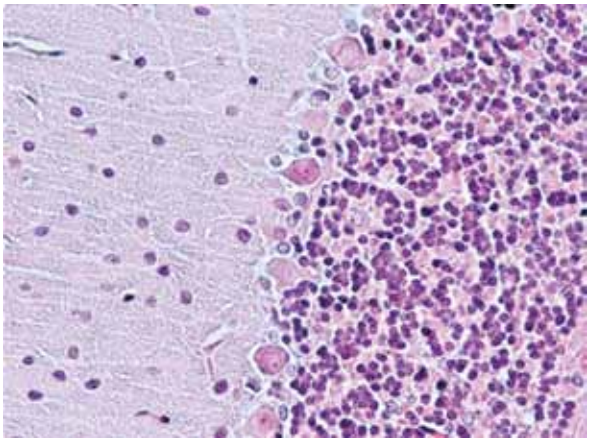

***Fig. 1.*** *Reversible changes in Purkinje cells of the cerebellum 24 hours after clozapine (150 mg/kg) administration. Hematoxylin-eosin. Magnification 400. Cell swelling, primary irritation*

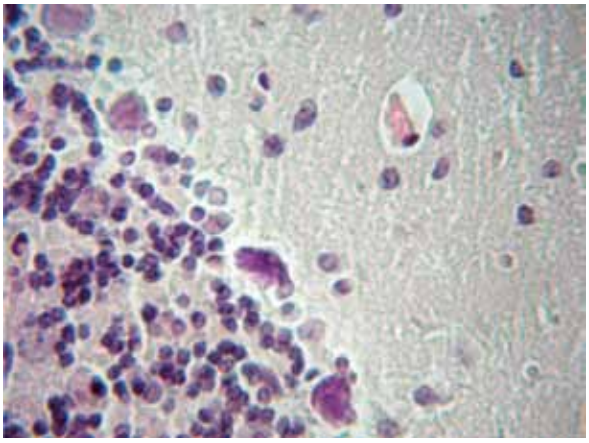

***Fig. 2.*** *Irreversible changes of Purkinje cells of the cerebellum 24 hours after clozapine (150 mg/kg). Hematoxylin-eosin. Magnification 400 Severe changes, cariocitolysis, "cells shadows"*

3 hours after combined clozapine and ethanol administration damage to Purkinje cells is detected (37–55% — reversible, 40–60% — irreversible).

24 hours after combined clozapine and ethanol poisoning 25–30% of the damage was reversible and 60–70% was irreversible. The absence of Purkinje cells in significant areas of their localization was also found. There was a decrease in the intensity of Nissl staining of the substance up to its complete disappearance.

The combination of clozapine and ethanol has a damaging effect on Purkinje cells. Previous studies show the development of dystrophic changes in cortical neurons [9]. Mechanisms of clozapine toxicity are shown in a number of studies [10]. Clozapine causes damage to myocardial and lung cells [1, 11, 12]. In case of combined clozapine and ethanol poisoning the damage to organs is more significant. After administration ethanol is converted to acetaldehyde which damages cell membranes. Ethanol and acetaldehyde inhibit the energy metabolism of cells by damaging the mitochondria. Moreover, they activate lipid peroxidation. In addition, cell damage is aggravated by the activation of lysosomal hydrolytic enzymes. The mechanisms of damaging effect of ethanol include the release of catecholamines. An important component of the pathogenesis of damage caused by ethanol is the effect of catecholamines and violation of hemomicrocirculation [12, 13]. In addition, clozapine damages mitochondria [14], affects the functional activity of astrocytes and microglia [15], and reduces the content of ceramide and sphingomyelin in the liver [16].

## CONCLUSION

Clozapine poisoning severely affects the Purkinje cells of the cerebellum, which is confirmed by an increase in the number of neurons that register signs of both reversible and irreversible damage. Purkinje cell alteration increases by 24 hours from the beginning of the experiment.

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# HISTOMORPHOMETRIC PARAMETERS OF THE CARDIAC CONDUCTION SYSTEM AND THE MYOCARDIUM: CORRELATING RESULTS OF POSTMORTEM FORENSIC ANALYSIS ON ALCOHOLIC CARDIOMYOPATHY AND CORONARY HEART DISEASE

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**ABSTRACT** — The article presents results of a study on the correlation of histomorphometric parameters of the heart in case of death from alcoholic cardiomyopathy (ACM), ischemic heart disease (IHD) and mechanical injury (control group) by the method of correlation analysis. The previous studies show that normally with age, the myocardium of the sinoatrial node (SAN) is most worn out and fibrosed. In case of IHD the His bundles are most worn out and fibrosed. In case of ACM, histomorphological changes are uniform in all areas of the myocardium. This indicator can be used in practice when providing differential diagnosis of the above-described types of pathology and causes of death. In addition, the results of our study confirm that myocardial lipomatosis is more typical of ACM, which increases its diagnostic value.

**KEYWORDS** — cardiac conduction system, alcoholic cardiomyopathy, ischemic heart disease, forensic histology, correlation analysis.

## INTRODUCTION

Differential diagnosis of various diseases in order to establish the cause of death is an important issue of pathological anatomy and forensic pathology. Currently, a lot of individual signs and their complexes that are observed for various causes of death have been described, but only a few of them are absolutely specific [5].The signs of one pathological condition often differ from the signs of another, not in character, but only in the frequency of their occurrence and the degree of their severity. This greatly complicates the establishment of the cause of death during the autopsy and histological examination [4].

One of the methods to solve this problem is to assess the severity of a diagnostic signs. In order to increase the objectivity of the result it is advisable to use the morphometric method. To increase the reliability of the results, the data from other research methods, for example, biochemical and immunohistochemical are used [3–5].

According to modern scientific methodology, a systematic approach is considered to be optimal for differential diagnostics [3]. A system is most often understood as a set of elements that are related to each other, therefore the essence of the system approach is the real or mental division of the studied object into its parts and the study of these parts, their interaction and the behavior of the whole system in general [2, 7]. There are also functional systems, where the interaction of their parts is subordinated to certain functions. The systems of the body in normal condition have been studied for a long time, but in pathology their structure and behavior, as well as the properties and interaction of their elements, change in different ways in different nosological forms. Sometimes the very set of pathological processes is considered to be a system, since the processes are also related to each other [3]. In practice, first of all this is achieved, through a mathematical study of the relationships (correlations) between the measured parameters of the system as whole and its elements. Therefore, the first and main mathematical tool of systems analysis of pathology is correlation analysis, and then other mathematical research methods should be chosen based on its results [7].

### *Aims*

We aimed to study the correlation between the micromorphological parameters characteristic of

death from ischemic heart disease (IHD), alcohol cardiomyopathy (ACM) and mechanical injury (control group) and to use these data for more objectivity in diagnosis of these causes of death in accordance with the requirements of evidence-based medicine.

The study of the age dynamics of the studied parameters was of particular interest, since it is impossible to estimate it by the methods of descriptive statistics or by comparing the groups. One can only compare different age intervals, which is methodologically incorrect, because the parameters do not change *in leaps*. Only correlation analysis makes it possible to investigate age dynamics [6].

## MATERIAL AND METHODS

105 cases were performed (for detailed characteristics, see Table 1). The diagnosis of IHD or ACM was established by macro- and micromorphological signs. For the control group we selected cases of rapid death from mechanical trauma in the absence of signs of IHD, ACM and cardiac trauma.

***Table 1.*** *Characteristics of the studied materials*

| Cause of death | IHD | ACM | Trauma |
|---|---|---|---|
| Age | 35–85 years old | 29–58 years old | 18–28 years old |
| Ethanol in blood | 0‰ | 0,5–1,0‰ | 0‰ |
| Ethanol in urine | 0‰ | 0,3–1,5‰ | 0‰ |
| Number of cases | 41 (women – 14, men – 27) | 40 (women – 8, men – 32) | 24 (men – 22, women – 2) |
| Total | 105 cases | | |

The sinoatrial node (SA or SAN) was singled out according to the generally accepted method [1], and a sample of the myocardium was also taken from the interventricular septum and His bundles. Standard histological and computerized histostereometric studies of these structures were carried out.

The following parameters were measured in all the structures:

1) the diameter of 10 cardiomyocytes in 3 fields of vision in each section at x400 magnification, then the average diameter of the cardiomyocyte in the structure was calculated;

2) the average area of connective tissue structures (fibrosis) outside the areas of postinfarction cardiosclerosis in 3 fields of vision at x400 magnification (area of fibrosis in 3 fields of view/total area of 3 fields of view);

3) the average area of lipomatosis in 3 fields of vision at x400magnification (area of lipomatosis in 3 fields of view/total area of 3 fields of view).

At the first stage, the statistical processing of the data was carried out using the methods of descriptive statistics. Then the closeness of the distribution of each parameter to normal was determined using the indices of asymmetry and kurtosis, as well as by comparing the mode, median and arithmetic mean. The distribution of all the studied parameters was close to normal; therefore, at the final stage, we used the calculation of the parametric correlation coefficients according to Pearson [6].

## RESULTS AND DISCUSSION

The obtained correlation coefficients between the studied parameters are presented in Table 2.

In case of death from trauma (conditional norm), no strong correlation was found between any of the studied parameters. A positive correlation of average strength was noted for the following pairs of parameters: the share of fibrosis in the SAN and the share of lipomatosis in the SAN; the proportion of fibrosis in the contractile myocardium and the share of lipomatosis in it; the share of fibrosis in the contractile myocardium and the share of SAN lipomatosis. In addition, the share of lipomatosis in the contractile myocardium was found to be related with the share of lipomatosis of the SAN and His bundle. When comparing these data with the information about the average values of these parameters, it becomes clear that fibrosis and lipomatosis of the myocardium of different departments are normally insignificant, especially in the area of the His bundle, and tend to grow in parallel to each other. Meanwhile, lipomatosis grows evenly in all departments. This is probably due to the early forms of cardiac pathology, which are usually not yet recorded during routine histological examination.

The proportion of SAN lipomatosis is related with the average diameter of the cardiomyocyte in His bundle. This possibly reflects compensatory hypertrophy of cardiomyocytes of the underlying parts of the conducting system during structural changes in the SAN that can disturb its function. Then it implies that lipomatosis disturbs the function of the elements of the conducting system more severely than fibrosis, which is understandable taking into account the pronounced dielectric properties of adipose tissue. There were no other correlations of the average diameter of the cardiomyocyte in the studied sections with other parameters.

With age, only the share of fibrosis in the SAN and the average diameter of the cardiomyocyte increases.

In case of ACM, the picture is completely different. Most of the parameters correlate with each other moderately or strongly, all the correlations are positive.

***Table 2.*** *Correlation coefficients between the studied parameters*

| Parameter | Parameter | ACM | IHD | Trauma |
|---|---|---|---|---|
| Age | Mean diameter of CMC of SAN | -0,01915 | -0,07705 | 0,56015 |
| Age | Mean diameter of CMC of IVS | -0,1837 | -0,12895 | 0,068338 |
| Age | Mean diameter of CMC of HB | -0,10855 | -0,31091 | 0,189366 |
| Age | Share of fibrosis of SAN | 0,577441 | -0,0131 | 0,440411 |
| Age | Share of fibrosis of CM | 0,401407 | -0,22162 | -0,08205 |
| Age | Share of fibrosis of HB | 0,55489 | 0,336179 | 0,099953 |
| Age | Share of lipomatosis of SAN | 0,514922 | -0,33163 | 0,098228 |
| Age | Share of lipomatosis of CM | 0,694397 | -0,41735 | 0,086459 |
| Age | Share of lipomatosis of HB | 0,44691 | -0,36281 | 0,076928 |
| Mean diameter of CMC of SAN | Mean diameter of CMC of IVS | 0,432466 | 0,135603 | 0,213257 |
| Mean diameter of CMC of SAN | Mean diameter of CMC of HB | 0,624463 | 0,438395 | 0,092965 |
| Mean diameter of CMC of SAN | Share of SAN fibrosis | 0,06973 | 0,342186 | 0,289918 |
| Mean diameter of CMC of SAN | Share of CM fibrosis | 0,233645 | 0,033839 | -0,22381 |
| Mean diameter of CMC of SAN | Share of HB fibrosis | 0,132708 | -0,09977 | 0,104599 |
| Mean diameter of CMC of SAN | Share of SAN lipomatosis | -0,16196 | 0,068105 | 0,165477 |
| Mean diameter of CMC of SAN | Share of CM lipomatosis | 0,003739 | 0,062196 | -0,02811 |
| Mean diameter of CMC of SAN | Share of HB lipomatosis | 0,008463 | 0,290362 | 0,181051 |
| Mean diameter of CMC of IVS | Mean diameter of CMC of HB | 0,520119 | 0,272227 | 0,229797 |
| Mean diameter of CMC of IVS | Share of SAN fibrosis | -0,10524 | -0,11804 | 0,186659 |
| Mean diameter of CMC of IVS | Share of CM fibrosis | 0,06376 | 0,059361 | -0,10747 |
| Mean diameter of CMC of IVS | Share of HB fibrosis | -0,30426 | -0,2468 | 0,003386 |
| Mean diameter of CMC of IVS | Share of SAN lipomatosis | -0,27271 | 0,130516 | -0,11304 |
| Mean diameter of CMC of IVS | Share of CM lipomatosis | -0,17088 | 0,318842 | 0,159252 |
| Mean diameter of CMC of IVS | Share of HB lipomatosis | -0,06361 | 0,170291 | 0,053979 |
| Mean diameter of CMC of HB | Share of SAN fibrosis | -0,23683 | 0,092784 | 0,356616 |
| Mean diameter of CMC of HB | Share of CM fibrosis | 0,046677 | 0,377867 | 0,354733 |
| Mean diameter of CMC of HB | Share of HB fibrosis | -0,17172 | 0,022028 | -0,38942 |
| Mean diameter of CMC of HB | Share of SAN lipomatosis | -0,26734 | -0,11221 | 0,502604 |
| Mean diameter of CMC of HB | Share of CM lipomatosis | -0,13138 | 0,188885 | 0,339385 |
| Mean diameter of CMC of HB | Share of HB lipomatosis | 0,014732 | 0,235344 | 0,096292 |
| Share of SAN fibrosis | Share of CM fibrosis | 0,706444 | 0,438847 | 0,08339 |
| Share of SAN fibrosis | Share of HB fibrosis | 0,707119 | 0,337486 | 0,189159 |
| Share of SAN fibrosis | Share of SAN lipomatosis | 0,218152 | -0,08301 | 0,370806 |
| Share of SAN fibrosis | Share of CM lipomatosis | 0,435002 | -0,09602 | 0,012553 |
| Share of SAN fibrosis | Share of HB lipomatosis | 0,074173 | 0,170852 | 0,151901 |
| Share of CM fibrosis | Share of HB fibrosis | 0,634753 | 0,276595 | -0,30082 |
| Share of CM fibrosis | Share of SAN lipomatosis | -0,09279 | -0,08896 | 0,536248 |
| Share of CM fibrosis | Share of CM lipomatosis | 0,348407 | 0,080652 | 0,466173 |
| Share of CM fibrosis | Share of HB lipomatosis | -0,10567 | 0,123727 | 0,259238 |
| Share of HB fibrosis | Share of SAN lipomatosis | 0,262653 | -0,17877 | -0,04005 |
| Share of HB fibrosis | Share of CM lipomatosis | 0,436198 | -0,47464 | -0,13218 |
| Share of HB fibrosis | Share of HB lipomatosis | 0,182928 | -0,39382 | 0,062725 |
| Share of SAN lipomatosis | Share of CM lipomatosis | 0,685566 | 0,513181 | 0,370733 |
| Share of SAN lipomatosis | Share of HB lipomatosis | 0,509615 | 0,485938 | 0,221327 |
| Share of CM lipomatosis | Share of HB lipomatosis | 0,499242 | 0,669926 | 0,533854 |

***Note:*** *CM — cardiomyocytes, IVS — interventricular septum, CM — contractile myocardium, HB — His bundle; bold type indicates correlation coefficients from 0.3 to 0.6, bold type and italics indicate strong correlation (more than 0.6).*

In case of ACM the share of fibrosis and lipomatosis increase in all the studied areas, especially the proportion of lipomatosis of the contractile myocardium with age (the correlation with age is strong). It is quite natural that in this case they all correlate with each other, especially the share of lipomatosis in the SAN and the contractile myocardium (strong correlation). In particular, the shares of fibrosis in all departments are related with each other, and this correlation is also strong. There is a correlation between the share of lipomatosis of the contractile myocardium with the share of fibrosis in all three sections, but the correlation between the proportion of fibrosis and lipomatosis of the contractile myocardium is weak. In addition, the mean diameters of cardiomyocytes in all three areas, especially in the SAN and the His bundle, correlate with each other.

These data reflect the dynamics of myocardial reactions to the toxic effect of alcohol and its metabolites. These substances are capable of affecting both the vascular bed and the parenchymal elements of the heart. They reduce the value of each parameter separately (parameters that correlate with each other are considered uninformative), while not changing their differential diagnostic role in each particular case. If fibrosis, lipomatosis, and changes in the diameter of cardiomyocytes are significant and affect uniformly both the conductive and contractile myocardium, this is an argument in favor of the diagnosis of ACM.

At death from IHD, in contrast to the results described above, many correlation coefficients are negative. Particularly, only the share of fibrosis of the His bundle increases. At the same time the average diameter of the cardiomyocyte in the same area and the share of lipomatosis in all studied areas, decrease with age. In this case, the share of lipomatosis in different areas correlate with each other positively. There is also a positive relationship of average strength between the share of fibrosis of the SAN and the share of fibrosis in other departments. This result seems paradoxical, because IHD is a typical age-related disease, and pathological changes in it should increase with age. However, the facts have an explanation that is important for practical needs. Diffuse fibrosis affecting the conducting system, and lipomatosis, are not the characteristic signs for the most common forms of coronary artery disease and are caused by other reasons. Therefore they do not have positive age-related dynamics, and are also little related to each other. For ischemic heart disease, according to the data obtained, a typical predominant violation of the His bundle is typical, and the share of fibrosis of the His bundle correlates inversely with the proportions of lipomatosis of the same area and contractile myocardium. It is fibrosis, not lipomatosis, that is characteristic of IHD, which is understandable, given the metabolic characteristics of alcoholic intoxication in comparison with ischemic heart disease. The share of fibrosis of the His bundle in IHD increases with age in response to atrophy and death of cardiomyocytes. Therefore, the average diameter of cardiomyocytes in this area decreases with age. And, finally, there is a positive relationship of average strength between the average diameters of cardiomyocytes in the bundle of His and in the SAN, which obviously reflects compensatory hypertrophy of less damaged parts of the conducting system.

## CONCLUSION

Thus, the correlation analysis of the histomorphometric parameters of the heart helped to establish that normally, with age, the myocardium of the SAN is worn out and fibrosized most. In case of IHD the His bundles are most worn out and fibrosed. In case ACM, histomorphological changes are uniform in all areas of myocardium. This conclusion is not only of theoretical significance, it can be used in practice in the differential diagnosis of these causes of death. In addition, our data confirm that among the causes of death studied, myocardial lipomatosis is typical only of ACM, which increases its diagnostic value.

From a thanatogenetic point of view, our data make it possible to explain the mechanisms of death of elderly people with insignificant alcohol intoxication with ACM or with coronary artery disease through edema of worn-out structures of the conducting system, associated with both hemodynamic and toxic damaging factors.

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# ANTIBIOTIC SENSITIVITY OF CLINICAL ISOLATES AT OUTPATIENT UNIT IN TVER, RUSSIA: A COMPARATIVE STUDY

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**ABSTRACT** — Background Discovery of antibiotics opened a new era in the treatment of bacterial diseases. However, the microorganisms are able to adapt and resist the effects of the drugs.
Objective Our study is aimed to investigate the sensitivity of clinical specimens to antibiotics.
Methods Clinical samples of 280 case record forms were collected at departments of surgery, urology and otorhinolaryngology in an ambulatory clinic (Tver, Russia) during 2019. The results of microbiologically assessed isolates from pharynx, nose, ears, eyes, wounds, sputum and urine underwent statistical analysis.
Results The outcomes confirmed a general trend of reduced susceptibility of bacteria to antibiotics. The worst result was shown by protected Amoxicillin; practically no microorganisms were sensitive to it. Protected Cephalosporins Cephalosporins of IV generation and Imipenem were among the best, although not highly sensitive to all the pathogens.
Conclusion United efforts of all states are required to combat the growing antibiotic resistance. It is necessary to adhere to strict regulations on dispense of antibiotics in pharmacies and the use of antibiotics therapy.

**KEYWORDS** — antibiotics, sensitivity to antibiotics, bacterial infection, antibiotic resistance.

## INTRODUCTION

The discovery of penicillin was a real breakthrough in the treatment of infectious diseases, for which Alexander Fleming, Ernst Chain and Howard Flory received the Nobel Prize in 1945. Then a series of discoveries of antibiotics followed: streptomycin, tetracycline, chloramphenicol. By 1980, there were more than 100 different antibiotics. However, after 4 years of widespread use of penicillin, effectively untreatable infections appeared. Bacteria have accumulated special protective mechanisms and have developed resistance to antibiotics. [1, 2].

Currently, antibiotic resistance is defined by the World Health Organization (WHO) as a major global challenge that requires immediate joint action to solve it [3]. There are several priorities to combat the increasing resistance of bacteria to antibiotics. Among them: a restricted use of antibacterial drugs for upper respiratory infections; avoiding antibiotics facilitating microbial growth; choosing antibiotic treatment with the account of its resistance and safety; adequate dosage and period of the therapy; prescribing antibiotics in optimal pharmaceutical form. And finally: development and introducing new antibacterial chemotherapy drugs into the practice. But as it is seen on practice, in some time, quite promptly, antibiotic resistance occurs. However, the pharmaceutical industry is reluctant to develop new antibacterial drugs. [4, 5]. Therefore all existing antibiotics were divided into 3 groups: access, observation, and reserve [5]. This separation was made in order to maintain the sensitivity of bacteria to at least some drugs and thus save a human life.

Originality of our study lies in investigating local antibiotic resistance within one medical institution. The geographic location of Tver region is at the borderline to Moscow, which allows indirect use of portal https://amrmap.ru/ to estimate resistance of microorganisms to antibacterial drugs [6, 7]. However, Tver medical institutions do not participate in this federal program. Therefore, the task of a doctor is to prescribe an adequate treatment with the account of possible sensitivity of microorganisms, which may vary in the conditions of a hospital or an outpatient unit.

## OBJECTIVE

A comparative bacteriological study was aimed to evaluate bacteria isolated from clinical material and to get a general picture of sensitivity of most common bacteria to commonly used antimicrobials.

## MATERIALS AND METHODS

280 case record forms of clinical materials underwent microbiological assessment and were statistically processed. Isolated specimens of wound tissues, pharynx, nose, eyes, ears, as well as sputum and urine were collected during the appointments with surgeons, urologists and otolaryngologists at Outpatient Clinic N1 of Tver City Hospital N7 (Tver, Russia) in the course of 2019. Clinical samples were collected by

a swab and placed in the tubes containing AMIES transport medium (APEXLAB, China). The samples were delivered to the laboratory within 40 minutes of collection. Bacteriological assessment of the clinical isolates was carried out in selective and differential growth medium, such as Endo agar, salt egg yolk agar, blood agar. Classical microbiological methods for an anaerobic condition at the temperature of 37° C were employed. After isolation of a pure culture, morphological and biochemical identification was performed. We followed clinical recommendations „Defining the susceptibility of microorganisms to antimicrobic drugs"(2014) to classify their susceptibility according to international European System EUCAST. Susceptibility to antibiotics of the isolated strains was tested employing diffusion method with Oxoid disks, UK followed by calculation of the degree of deviation for zone diameters. Microsoft Excel 2016 was used for statistical analysis of the data.

## RESULTS AND DISCUSSION

The study revealed that Gr + microflora prevails in the overall picture of the microbiological profile, among the members of which Staphylococcus aureus (25.6%) and hemolytic streptococcus (17.1%) were most often grown. Among members of Gr-microorganisms, Escherichia coli were leading (21.9%).

We investigated the sensitivity of most frequent pathogens to antibiotics commonly used in medical practice. Our outcomes showed reduced sensitivity of bacteria E. Aerogenes to most antibiotics, especially to Amoxicillin/clavulanic acid (44.4%). This may be attributed to the fact that in more than 25% of cases this drug is prescribed for the treatment of ENT infection (analytical data of DSM Group). However, bacteria of this family showed rather high sensitivity to amikacin and cefepime (90.0%).

The strains seeded with E. Coli also proved to be less sensitive to ammoxicillin/clavulanate (36.8%). At the same time, their sensitivity to amikacin was rather low (59.7%), as well as third-generation cephalosporins: ceftriaxone and cefotaxime (62.5% and 66.7%, respectively). The highest antibacterial activity against Escherichia coli was found in fourth-generation cephalosporins: cefipim (100%) and protected cephalosporins: cefoperazole/sulbactam (100%).

Among all seeded pathogens, K. Pneumonia and P. Aeuruginosa displayed most multidrug resistance to antibiotics. Klebsiella pneumoniae was found to be most sensitive only to cefoperazole/sulbactam. The remaining antibiotics showed insufficient activity against seeded strains. Especially paradoxical this picture of resistance looks with respect to IV generation cephalosporins (cefepime — 61.5%), fluorinated quinolones (ciprofloxacin — 50.0%) and III generation aminoglycosides (amikacin — 42.9%). The complete resistance of Pseudomonas aeruginosa to amoxicillin / clavulanate and a sharp decrease in sensitivity to all cephalosporins, including protected ones, up to complete resistance to ceftriaxone, also look paradoxical. At a very high level, P. Aeuruginosa remains susceptible to amikacin, imipenem and ciprofloxacin.

***Table 1.*** *Sensitivity of surgical profile pathogens to antibacterial drugs (%)*

| | E. aerogenes | E. Coli | K. pneumonia | Pseudomonas aeuruginosa | S.aureus | St. pyogenes |
|---|---|---|---|---|---|---|
| Amikacin | 90,0 | 59,7 | 42,9 | 100 | 25,0 | 75,0 |
| Amoxicillin / clavulanate | 44,4 | 36,8 | 36,4 | 0 | 14,3 | 65,3 |
| Imipenem | 80,0 | 88,9 | 38,5 | 100 | 66,7 | 72,8 |
| Cefepim | 90,0 | 100 | 61,5 | 35,3 | 77,8 | 100 |
| Cefoperazone / sulbactam | 81,8 | 100 | 90,9 | 33,3 | 100 | 100 |
| Cefotaxime | 72,7 | 66,7 | 50,0 | 29,7 | 88,9 | 100 |
| Ceftriaxone | 75,3 | 62,5 | 46,1 | 0 | 88,9 | 76,4 |
| Ciprofloxacin | 78,2 | 71,4 | 50,0 | 78,1 | 88,9 | 61,8 |

A low sensitivity of Staphylococcus aureus to protected amoxicillin and amikacin is revealed. At the same time, it should be noted that the isolated strains remain sensitive to most antibiotics, especially to cephalosporins combined with β-lactamase inhibitors. A similar picture is observed for hemolytic streptococcus. It remains sensitive to most antibiotics, which allows the use of amoxicillin as the first choice in the

treatment of infections caused by this pathogen. Despite the high sensitivity to most antibiotics, it is not recommended to use cephalosporins of the last generations in these cases, due to the high risk of selection of microorganisms and the occurrence of multiresistance [5].

## CONCLUSION

Our results have confirmed a general tendency of increasing insensitivity of various bacteria to antibiotics. Possibly, horizontal transfer of antibiotic resistance genes plays an important role in this process [8, 9]. A pattern of multiresistance is observed in such strains as K. Pneumonia and P. Aeuruginosa, which cause dangerous bacterial diseases and can lead to death. In our study, Staphylococcus aureus and Hemolytic streptococcus have developed least resistance, which enables treating them with antibiotics of a narrower spectrum in order to inhibit the selection of microorganisms and develop resistance. Also, to inhibit the spread of multiresistance, the precautions of working with chemotherapy should be applied, namely: observe the duration of the course, the frequency of administration and dosage. It is recommended to restrict the use of antibiotics for preventive purposes, as well as to forbid self-prescription of antibiotics.

We reiterate the urgency of joint efforts of interdisciplinary medical teams on cross-national level on inhibiting the spread of antibiotic resistance and rationalizing the use of antibiotics at outpatient care.

## CONTRIBUTIONS

Authors contributed to the manuscript equally.

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# EVALUATION OF THE ORAL MICROBIOTA IN ENT AND DENTAL PATIENTS

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**ABSTRACT** — The World Health estimates oropharyngeal mycosis as the second most common fungal infections (40% of all cases). The study of microbiocenoses and their role in maintaining health and the development of pathology is a complex multifactorial problem which includes the study of not only the microbial communities themselves but also the components and metabolites of microflora as well as their interaction with a macroorganism considering personification and social factors. Our study was aimed to evaluate possible microbial scenery changes using microscopic examination of pharynx and oral cavity specimens from patients at departments of otolaryngology and dentistry of Polyclinic No. 1 (Tver, Russia). The investigation of samples of dorsal surface of patients' tongues (503 participants) was carried out between January 2018 and October 2020. The selection criterion met the patients with the following symptoms: swallowing difficulties, feeling of a "lump in the throat", a sore throat, a dry mouth, a burning tongue. Our findings showed the persistence of fungal infection of the oral cavity in 68,5% of cases with numerous otolaryngological and dental diseases. *Candida albicans* microbial associations with cocci as well as combinations with mixed microflora were identified in 45% of cases. Anatomical and functional aspects of the oral cavity account for an interdisciplinary approach to the treatment of such patients with a close cooperation between otolaryngologists and dentists. .

**KEYWORDS** — microbial scenery, pathogenic microflora, oropharynx, oral cavity, mucosa.

## INTRODUCTION

According to the data, provided by the World Health Organisation, one fifth of the world's population suffers from various fungal infections, the second most common of which (40% of all cases) is oropharyngeal mycosis. Its main causative agent (*Candida albicans*) is an opportunistic microorganism that is a part of the normal microflora of the mucosa of the gastrointestinal and urogenital tract. Oropharyngeal mycosis is accompanied by a burning and dry tongue, a bad taste in the mouth and discomfort while swallowing. Oral cavity, which determines the state of human health, constitutes a comprehensive, unique microecological system, closely linked both to the internal environment of an organism as well as to the external one [1, 2].

The study of microbiocenoses and their role in maintaining health and the development of pathology is a complex multifactorial problem which includes the study of not only the microbial communities but also the components and metabolites of microflora as well as their interaction with a macroorganism considering personification and social factors. It is known that in terms of quantitative and qualitative diversity, the oral mucosa is a fairly extensive biotope, and bacteria demonstrate specific tropism in relation to various anatomical oral cavity surfaces. Teeth, gums, gingival fluid, gingival grooves, the mucosa of the tongue, cheeks, hard and soft palates, ducts of salivary glands with their saliva have significant differences in the microbial communities composition, as well as the existence of a separate tonsillar microbiome [3]. The oral cavity microbiota determines colonisation resistance, local immune resistance and the microecosystems formation not only of its own biotope, but also of the entire gastrointestinal tract, the biotope of the broncho-pulmonary system [4].

The purpose of the study was to evaluate possible changes of microbial scenery using microscopic examination of pharynx and oral cavity samples from ENT and dental patients.

## MATERIAL AND METHODS

The evaluation of clinical specimens taken from the dorsal surface of patients' tongues (503 participants) was carried out directly in Polyclinic No. 1 of Tver State Clinical Hospital No. 7 (Russia), between January 2018 and October 2020. Patients for participation at the research were selected by the following complaints: swallowing difficulties, a feeling of a "lump in the throat", a sore throat, a dry mouth, a burning tongue. 503 patients took part in the investigation: 18,84% of them were male and 81,16% were female, both in the age between 18 and 80 years old. The clinical material from the tongue surface was captured on the slide with a sterile cotton, followed by Gram-staining and a microscopic research. The finding were

systematized and processed with the licensed software Microsoft Excel 2016.

## RESULTS AND DISCUSSION

As a result of the microscopic examination of oral cavity and pharynx samples in 503 patients mycotic mycelium filaments and fungal bodies were detected in various amounts in 68,59% (345 patients). A negative result of detecting mycotic filaments and mycelium was observed in 31,41% (158 patients). Variations in the number of fungal bodies also occurred in their combination with bacteria (45,72%), among which associations of fungi and representatives of the cocco-diplococcal flora accounted for 20,48% (103 cases), and the combination of fungi and representatives of mixed flora were found in 22,267% (112 cases). It should be noted that a combination of an insignificant amount of coccal flora and single elements of the fungus were found in 2,98% (15 cases). Fungal mycelium filaments, both single and in the form of myco-bacterial associations were identified in 14,71% (74 cases). Individual elements of the fungus and their associations were detected in 2,98% (15 cases), elements of the fungus and their associations — in 25,65% (129 cases), elements of the fungus + and their associations — in 16,30% (82 cases), elements of the fungus +++ and their associations — in 8,95% (45 cases), Table 1.

The outcomes of our research, in 68,5% of all cases of various otolaryngological and dental diseases revealed the persistence of fungal infection of the oral cavity. In 45% there were Candida albicans microbial associations with cocci (the genus *Streptococcus*, the genus *Staphylococcus*) as well as combinations with mixed microflora (representatives of the families *Pseudomonadaceae* and *Enterobacteriaceae*, the genus *Corynebacterium*, the genus *Clostridium*, the genus *Lactobacillus*, the genus *Rothia*, the genus *Actinomyces*). It should be noted that the identified microorganisms in conjunction with the anamnesis data, complaints and objective examination may indicate various types and degrees of oral cavity and oropharynx microbiome disorder. The obtained data correspond to the data of literature sources on the presence of the phenomena of oral cavity and oropharynx microbiome simbiosis and commensalism.

According to the literature, 99% of bacteria exist in the form of biofilms attached to the substrate, and they are more resistant to the immune system factors and antibiotics. The reason for this phenomenon is simple - bacteria included into the substrate in the form of biofilms acquire qualitatively new properties [5]. An interesting fact is that synergistic interactions between fungi of the genus Candida albicans and commensal streptococci exert an important role in

***Table 1.*** *Microbial scenery variation of the investigated material*

| The results of microscopic research | % | Absolute quantity |
|---|---|---|
| Mycelium of the fungus was not revealed | 20,67 | 104 |
| Fungus mycelium filaments | 3,38 | 17 |
| Funguselements + | 11,94 | 60 |
| Fungus elements ++ | 4,57 | 23 |
| Fungus elements +++ | 2,98 | 15 |
| Insignificant coccal flora, isolated elements of the fungus | 2,98 | 15 |
| Moderate coccal flora, fungus elements were not detected | 2,18 | 11 |
| Abundant coccal flora, fungus elements were not detected | 4,37 | 22 |
| Abundant coccal-diplococcal flora, fungus elements + | 11,54 | 58 |
| Abundant coccal-diplococcal flora, fungus elements ++ | 6,36 | 32 |
| Abundant coccal-diplococcal flora, fungus elements +++ | 2,58 | 13 |
| Abundant mixed flora, fungus elements were not detected | 4,17 | 21 |
| Abundant mixed flora, fungal mycelium | 11,34 | 57 |
| Abundant mixed flora, fungal elements + | 2,19 | 11 |
| Abundant mixed flora, fungal elements ++ | 5,37 | 27 |
| Abundant mixed flora, fungal elements +++ | 3,38 | 17 |
| Total number of carried out studies | 100 | 503 |

the transition of the relationship from commensalism to pathogenetic impact on oral mucosa, causing deep damage. At the same time it is important to emphasize the fact that in this case streptococci grow in close contact with the oral mucosa while fungi grow on the bacterial surface [6]. Researchers distinguish resident (permanent), additional (transient) and random microflora. On the oral mucosa among bacterial spectrum prevail microorganisms belonging to the genus *Streptococcus* (*S. viridans*, *S. pyogenes*, *S. pneumoniae*, *S. oralis*), the genus *Staphylococcus* (*S. aureus*, *S. epidermidis*, *S. hominis*), *Corynebacterium* (*C. tuberculostearicum*, *C. pseudodiphtheriticum*, *C. aurimucosum*, *C. amycolatum*, *C. durum*, *C. afermentaslipophilum*, *C. minutissimum*, *C. urealyticum*, *C. propinquum*), *Candida albicans* and *Actinomyces viscosus* are identified in a single quantity [1, 2, 3]. In the structure of palatine tonsils microbial scenery α- and γ-hemolytic streptococci (*S. parasanguinis*, *S. mitis*, *S. salivarius*, *S. Oligofermentans*), gram-positive bacteria of the genus *Rothia* (*R. mucilaginosa*, *R. dentocariosa*) dominates. Among the microorganisms of the genus *Actinomyces spp. oral* and the genus *Staphylococcus aureus*. There are also bacteria belonging to the genus *Corynebacterium* (*C. accolens*, *C. tuberculostearicum*) in the biotope of tonsils.

With a change in the quantity and quality of the resident microflora composition, oral functional fullness and anatomic integrity disorders, the residents of the transient microflora actively form etiopathogenetic links in the occurance of pathologic processes leading to the oral mucosa dysbiosis. This means that the oral microflora, as a highly sensitive indicator, reacts with qualitative and quantitative shifts to changes in the state of organs and systems of the human body as a whole.

## CONCLUSIONS

Fungal flora is prevailing in oral cavity area of ENT and dental patients, which have to be subjected to a specific antifungal therapy to stimulate the growth of oral and oropharynx microflora. Due to anatomical and functional aspects of the oral cavity and pharynx, the search of treatment solutions for such patients requires an interdisciplinary approach and a close cooperation between otolaryngologists and dentists.

### *Author Contributions*

Authors contributed to the manuscript equally.

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# PERSONALIZED CHOICE OF OPIOID THERAPY IN A PATIENT WITH CHRONIC PAIN SYNDROME ON THE BACKGROUND OF PANCREAS CANCER: CLINICAL CASE REPORT

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**ABSTRACT** — The paper presents a successful experience of personalized choice of opioid therapy when taking into account genetic and nongenetic factors in a patient with chronic pain syndrome on the background of pancreas cancer.

**KEYWORDS** — opioids, case study, pancreas cancer, chronic pain syndrome, personalized medicine.

A 65-year-old patient A. complains of weakness, increased fatigue, persistent pain in the upper abdomen 2 p. by VRS with intensification when changing the position of the body up to 3 p.; yellowness of the skin; reduction of the duration of night sleep to 4–5 hours.

*Medical history:*

The patient notes the appearance of jaundice within two weeks. According to the examination results, the patient was diagnosed with malignant neoplasm of the head of the pancreas 4 tbsp T3NxM1 (C25.9). Obstructive jaundice. After additional examination, the patient underwent external drainage to decompress the biliary tract.

After 3 weeks, a trial laparotomy with biopsy of the lymph nodes of the abdominal cavity was performed in order to clarify the staging and verification of the process. Abdominal carcinomatosis, ascites, chronic pain syndrome 3p on a verbal rating scale were added to the structure of the existing diagnosis.

By the decision of the medical commission, the patient was assigned palliative status, taking into account the fourth clinical group, and it was recommended to carry out systemic chemotherapy on an outpatient basis.

*Anamnesis of life:*

For five years he has been suffering from type II diabetes mellitus, non-insulin dependent. Ten-year history of hypertension 3st., risk 4., ischemic heart disease 2 functional class. The patient suffers from a duodenal ulcer with rare exacerbations (H. p. -). He is constantly taking antihypertensive, hypoglycemic drugs.

*Objective status:*

The patient's condition is satisfactory. Yellowness of the sclera and skin. There was no peripheral edema. The number of respiratory movements per minute is 19, breathing is hard, the absence of wheezing. The heart sounds are muffled, the rhythm is correct, the emphasis of the second tone is on the aorta, the number of heartbeats is 84 per minute. Blood pressure is 145/89 mm Hg. The tongue is coated with white bloom. The abdomen is soft. Percussion sizes of the liver are not increased.

The spleen is not enlarged by palpation. A slight soreness in the right mesogastrium is determined. Symptom XII ribs are negative on both sides. Of the features of the local status: on the right in the intercostal space is drainage, liquid discharge of yellow up to 800 ml per day. The stool is liquid with foam impurities. The results of laboratory and functional studies are shown in Table 1.

Against the background of systemic chemotherapy with capecitabine 2000–2500 mg/m$^2$/ a day, orally from day 1 to day 14 every 3 weeks ECOG status 3 points, BMI 24.16 kg/m$^2$, chronic pain intensity 3 points on a verbal rating scale and 7/10 points on a digital rating scale were observed. The patient was prescribed oral morphine sulfate in a daily dose of 90 mg in two doses on an outpatient basis, ketoprofen 100 mg intramuscularly at 22.00 and 100 mg rectally at 8.00 and 15.00, amitriptyline 12.5 mg at 23.00.

***Table 1.*** *Laboratory and functional indicators*

| Markers | Determined values | Reference values |
|---|---|---|
| Total protein,g/l | 56,7 | 64–83 |
| Potassium,mmol/l | 3,15 | 3,5–5,1 |
| Amylase,E/l | 27 | 28–100 |
| Creatinine,mkmol/l | 56 | 44–80 |
| ALT,E/l | 22,3 | 5–33 |
| AST,E/l | 35,2 | 5–32 |
| Glucose, mmol/l | 7,10 | 4,11–5,89 |
| Urea, mol/l | 2,30 | 2,76–8,07 |
| Total bilirubin, μmol/l | 14,5 | 5–17 |
| ESR, mm/hour | 22 | 2–18 |
| CA 19, ED/ml | 988,81 | 0–27 |
| GFR, ml/min/1.73 $m^2$ | 89 | ≥ 60 ml / min - a sign of preserved renal function |
| Child-Pugh scale score, points | 5 | Class A — 5–6<br>Class B — 7–9<br>Class C — 10–15 |
| BMI, $kg/m^2$ | 34 | 18,5–24,99 |
| ECOG, points | 2 | 1–4 |
| MMSE, points | 28 | 28–30 points — no impairment of cognitive functions |
| ESAS, points | 3 | |
| DN 4, points | 3 | ≥4 points — a sign of neuropathic pain |

***Abbreviations:*** *ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI — body mass index; GFR — glomerular filtration rate; ESR — erythrocyte sedimentation rate; CA19 — tumor marker; DN4 (Douleur Neuropathiqueen 4 questions) — a questionnaire to identify the neuropathic component of pain; ECOG (Eastern Cooperative Oncology Group) — physical status assessment scale; ESAS (Edmonton Symptom Assessment System) — Edmonton Symptom Assessment Scale; MMSE is a short scale for assessing mental status.*

After 3 weeks of therapy with a gradual increase in the patient, the development of morphine-associated constipation and an increase in the intensity of chronic pain syndrome up to 4b on a verbal rating scale and 8–9b on a digital rating scale were recorded.

Prescribing trimebutin in a daily dose of 400 mg and lactulose 60 ml a day did not give the desired result. The assessment of the reliability of the relationship between morphine sulfate and the development of constipation on the Naranjo scale was 9 points (definitely), a notification about the development of an adverse reaction was filled out.

A detailed analysis of comorbidity pharmacotherapy did not reveal constipation risk factors.

On the recommendation of a clinical pharmacologist, the patient underwent pharmacogenetic testing for carriage of SNV rs2032582 and rs1045642 of the *ABCB1* gene, rs1800795 of the *LOC541472* gene.

The analysis of the possibility of a personalized choice of opioid therapy is realized thanks to the created software-analytical complex "Evolutionary algorithm for the automated formation of decision support models for predicting the safety of opioid therapy" (authors Lipinsky L.V., Polyakova A.S., Melnikova O.D., Bobrova O. P., Schneider N.A., Zyryanov S.K., Petrova M.M., Russia, Krasnoyarsk).

The basis for the creation of this algorithm was the clinical, laboratory and genetic factors of patients - residents of Eastern Siberia with chronic pain syndrome against the background of pancreas cancer.

As part of the development of models for predicting the safety of opioid therapy, nine different machine learning methods were used with the resulting selection of the best quality model.

The best quality model was characterized by the use of at least fifty runs used by fifty-seven traits of patients with pancreas cancer.

An example of the selection of significant predictor factors for the implementation of constipation in patients with pancreas cancer based on the results of machine learning is presented in Table 2.

***Table 2.*** *Comparative significance of the signs of the prognostic model of constipation "Decision trees for the classification problem" in the group of patients with pancreas cancer by the example of morphine sulfate*

| Sign | Significance |
|---|---|
| AST0 | 0,296 |
| Ascites | 0,0013 |
| Pancreas head | 0,0022 |
| Stage _bin | 0,02 |
| CC rs1800795 gene *LOC541472* | 0,0008 |
| GG rs2032582 gene *ABCB1* | 0,007 |
| AA rs1045642 gene *ABCB1* | 0,0013 |

***Abbreviations:*** *AST0- aspartate aminotransferase; Pancreas – pancreas.*

According to the results of genetic testing, this patient turned out to be a carrier of the homozygous genotype GG of the single nucleotide variant rs2032582 of the *ABCB1* gene, CC of the single nucleotide variant of rs1800795 of the *LOC541472* gene and AA of the single nucleotide variant of rs1045642 of the *ABCB1* gene.

And also the fourth stage of the process, the localization of cancer in the head of the pancreas, the presence of ascites and the level of aspartate aminotransferase with a cut-off point of more than 31.5 U/L confirmed the significance of a mutually aggravating prognostic effect for the obligatory realization of morphine-associated constipation. The patient also did not have prognostic factors for predicting the realization of morphine- and fentanyl-associated neurotoxicity and pharmacoresistance according to the results of the automated testing. It should be borne in mind that modeling using machine learning methods predetermines the mutually influencing associative nature of significant prognosis factors, in contrast to the monocomponent influence of each factor separately in a particular patient. Thus, taking into account the hepato-renal functional reserve of the patient, nutritional status, obtained predictive results, clinical data, structure and volume of concomitant therapy for analgesia as part of the combined treatment, fentanyl TTS was prescribed taking into account the conversion rate. The choice of the drug is explained by the lack of noroxycodone and pure oxycodone in Russia. This choice of opioid therapy was also predetermined by the difficulties in providing oxycodone/naloxone in the Krasnoyarsk region. Further monitoring of the patient's condition showed a decrease in the intensity of chronic pain to 1b on a verbal rating scale at rest and 2b during movement using 100 μkg fentanyl TTS (the dose was titrated in increments of 25 μkg once every 72 hours for 14 days) with no constipation.

This example clearly demonstrates the possibility of a comprehensive assessment of the combination of various most significant predictive factors for the implementation of this adverse reaction.

Thus, the use of an optimal set of seven prognostic factors (clinical — genetic and laboratory) for the selection of a priori personalized non-invasive opioid therapy made it possible to increase the efficiency and safety of the treatment in a palliative patient.

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# EFFECT OF ZINC SULFATE ON BASIC CLINICAL AND LABORATORY MARKERS OF DIABETES

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**ABSTRACT** — The work is devoted to evaluating the effect of zinc sulfate on the basic clinical and laboratory markers of diabetes mellitus against the background of hypoglycemic therapy with metformin hydrochloride. The study was conducted on volunteers — patients with type II diabetes mellitus. Changes in carbohydrate metabolism were studied by evaluation of blood glucose levels, C-peptide and insulin levels, glycated hemoglobin. It was found that the use of zinc drugs helps to reduce the indicators of the clinical and laboratory markers. The changes of these indicators in men were more pronounced compared to women. The supplementation of zinc sulfate into the treatment regimen of patients with type II diabetes mellitus contributes to a more effective reduction in the indicators of carbohydrate metabolism compared to the conventional therapy.

**KEYWORDS** — type II diabetes mellitus, hypoglycemic therapy, zinc sulfate, markers of diabetes mellitus.

## INTRODUCTION

Today type II diabetes mellitus is an important problem in endocrinology and medicine, which is associated with the widespread prevalence of this disease. It is noted that this endocrine pathology is socially significant, as it leads to an increase in the percentage of disability and death of patients from developing complications. In this connection, the development of new methods for the correction of this disease is an urgent task [3, 7].

A major treatment of type II diabetes is the use of oral hypoglycemic agents, mostly the drugs of the class of biguanides and thiazolidinediones. However, their use does not always result in positive dynamics. Furthermore, clinical studies have noted a high percentage of complications, which are especially pronounced in elderly patients. In this connection, the use of additional agents that help reduce blood glucose levels is a promising direction [2, 4, 5].

Zinc plays a significant role in the development of diabetes II. It was found that insulin is synthesized and stored in β-cells of the pancreas in the form of $Zn^{-}$ insulin crystals. It has been proven that in the cofactor $Zn^{2+}$ is involved in the processing and storage of insulin and is also a signaling molecule for α-cells, being released into the extracellular space after secretion, insulin plays the role of an inhibitor of glucagon secretion by directly triggering ATP-sensitive potassium channels. It has also been shown that patients with diabetes mellitus have a deficit of zinc, especially with a chronic complicated course [1, 6, 8].

Hence, ***the purpose of this work*** was to study the effect of zinc sulfate on the main clinical and laboratory markers in patients with type II diabetes mellitus.

## MATERIALS AND METHODS

The studies were conducted with the participation of 36 volunteers — patients with type II diabetes mellitus aged 55 to 65 years (20 women and 16 men). In all patients this diagnosis was made 5 years ago and at the time of the study all of them were taking metformin hydrochloride as a hypoglycemic agent (Siofor 850; Berlin-Chemie AG/Menarini Group, Germany).

All participants were divided into 3 groups. The participants of the first control group were given metformin hydrochloride 850 mg only once a day. The second group comprised of women who received metformin hydrochloride 850 mg per day and zinc sulfate 124 mg (Zincteral; Teva, Israel) twice a day. The third group consisted of men who received the same treatment as women. Evaluation of the main clinical and laboratory markers of diabetes mellitus (blood glucose, C-peptide and insulin levels, glycated hemoglobin) was carried out 2 months after the start of treatment.

The results of determining the main clinical and laboratory markers of diabetes mellitus are presented in the table.

The glucose level of blood in the group of men who received zinc sulfate along with metformin hydrochloride was lower by 20%, in the group of women — by 14% compared with the group of patients receiving the standard treatment. Glycated hemoglobin decreased in both groups by 15%. As a result of the use of zinc sulfate in patients with type II diabetes mellitus

***Table 1.*** *The level of the main clinical and laboratory markers of diabetes mellitus*

| Groups of patients | Indicators | | | |
|---|---|---|---|---|
| | Blood glucose; Mmol/l | Glycated hemoglobin; % | C-peptide; ng/ml | Insulin; μU/ml |
| Control (metformin hydrochloride) | 7,3 ±1,2 | 7,6±0,9 | 5,3±0,7 | 21,9±1,8 |
| Men (metformin hydrochloride + zinc sulfate) | 5,9±0,9 | 6,4±0,9 | 3,3±0,5 | 7,6±1,4 |
| Women (metformin hydrochloride + zinc sulfate) | 6,3±1,1 | 6,6±0,8 | 3,5±0,9 | 8,3±1,5 |

there was a decrease in the level of C-peptide in the male group by 38% and in the female by 34%; insulin decreased by more than 60% compared to the group of patients treated with metformin hydrochloride alone.

Taking into account our outcomes it can be assumed that supplementary intake of zinc preparations facilitates a decrease in clinical and laboratory indicators of diabetes mellitus and its compensation. It is interesting that the positive changes in these indicators in men were more pronounced as compared to women, which may be attributed to the level of testosterone and a more developed muscular system [8].

## CONCLUSION

Thus, supplementation of zinc sulfate in the management of patients with type II diabetes mellitus contributes to a more effective decrease in indices of carbohydrate metabolism in comparison with the standard therapy.

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# SPECTROMETRY AS A NEW INSTRUMENTAL METHOD FOR VERIFICATION OF SALIVA CRYSTALLOSCOPIC STUDY

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**ABSTRACT** — The aim of the study was to evaluate the spectrometric characteristics of crystalloscopic facies of saliva in practically healthy people. We studied own crystallization of mixed saliva from 65 healthy adults by classic crystalloscopy (with parametric criteria) and spectrometry (at wavelengths of 300, 350 and 400 nm). Based on the study, the standard spectrometric characteristics of dried saliva samples from practically healthy adults were established. The obtained spectrometric patterns of saliva crystalloscopic facies can be used as reference intervals for a wide range of salivadiagnostics tasks.

**KEYWORDS** — saliva, crystallization, facia, spectrometry, biocrystallomics.

## INTRODUCTION

Recently, the scientific community has become increasingly interested in oral fluid as a material for studying its capacity to specific dehydration structuring [1–3, 5, 7]. In recent decades, its study has been considered as an integral test that provides generalized information about the composition and properties of this biological liquid [1–3, 7]. In particular, the study of saliva microcrystallization in dentistry is highly informative [2, 4, 5, 7]. The largest number of works is related to the diagnosis and treatment of patients with caries, including monitoring the effectiveness of correction technologies [7]. In addition, the features of the physical and chemical properties of artificial saliva have been studied using microcrystallization technologies [4]. At the same time, there is a question of objectifying the results of evaluating the crystallogenic properties of saliva, which can be solved by applying instrumental methods.

In this regard, ***the aim of the study*** was to evaluate the spectrometric characteristics of crystalloscopic facies of saliva in practically healthy people.

## MATERIAL AND METHODS

Mixed saliva was obtained in 65 healthy adults without any dental pathology (age 24–27 years). Oral fluid was collected in the morning (9–10 am) in a well-lit room. During the 3 hours before the study, the subjects did not perform significant physical activity and were not in a state of psychoemotional stress. Before collecting the biological fluid, the subjects thoroughly rinsed their mouths with 100 ml of distilled water for 5 minutes. Then the oral fluid (amount – 1 ml) was collected by spitting into clean, dry test tubes.

Further, the microspecimens (facias) were prepared according to the method of classical crystalloscopy [6]. The results of structuring were evaluated using a previously developed system of semi-quantitative parameters (specifically — crystallizability and structure index) [6]. At the next stage, all samples of biological fluid were investigated by spectrometric analysis performed on a spectrophotometer "PowerWave XS" (USA) at wavelengths of 300, 350 and 400 nm. To level the effect of glass characteristics on the results of spectrometric studies of saliva facies, a correction for the optical density of glass was introduced.

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

## RESULTS

At the first stage, the features of saliva self-crystallization in the formed group of practically healthy people were evaluated (Fig. 1). It was found that the level of the main parameters of the crystalloscopic test in the examined individuals lay in the previously selected normal range [2, 6]. At the same time, the crystallizability and structure index, which characterize the density of crystal elements and the complexity of their structure, respectively, were determined at high values.

The second stage of analysis of crystalloscopic facies was their direct spectrometric study (Fig. 2). It is shown that the spectrometry analysis of saliva crystallograms allows forming a stable *pattern* for all used wavelengths (300, 350 and 400 nm).

The study of the optical density of the preparations in the near-visible range of the spectrum

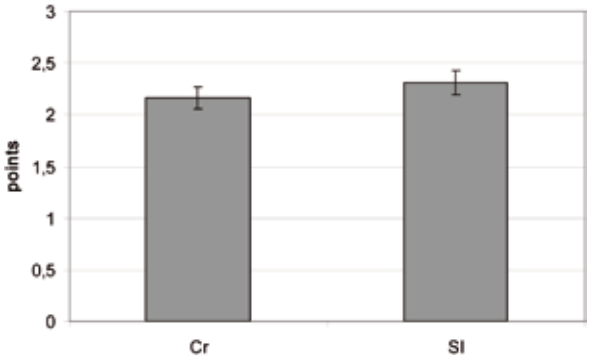


*Fig. 1. Crystallizability (Cr) and structure index (SI) values in dried specimens of mixed saliva of healthy adults*

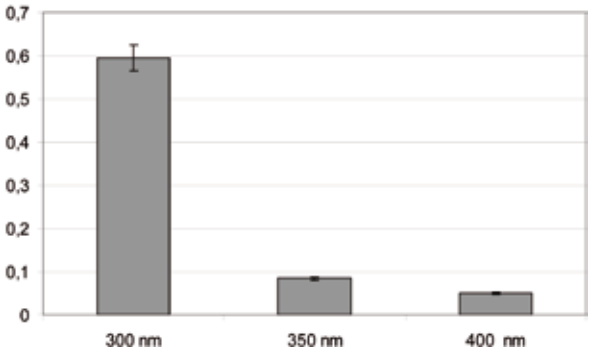


*Fig. 2. Optic density of crystalloscopic faicas of mixed saliva of healthy adults (at different wavelengths)*

(400 nm) allowed us to detect its approach to zero. In addition, a direct correlation was found between the crystallizability and the structure index levels with the optical density of facies at a wavelength of 300 nm (r=0.68 and 0.57, respectively, p<0.05).

## CONCLUSION

Based on the study, the standard spectrometric characteristics of dried saliva samples from practically healthy adults were established. The obtained spectrometric patterns of saliva crystalloscopic facies can be used as reference intervals for a wide range of salivadiagnostics tasks.

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# PROINFLAMMATORY AND ANTIAPOPTOTIC MARKERS OF THE STAGES OF ACUTE ENTERAL INSUFFICIENCY

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**ABSTRACT** — The diagnostic significance of the CD3 and Bcl-2 markers for determining the stage of acute intestinal failure was investigated. The authors used their own model. Loperamide at a dose of 0.09 mg/kg/day was used as a drug inducing intestinal failure. The study was carried out on 36 male Wistar rats weighing 250±30 g, comparable in age. The study groups included 12 animals. The length of the villi, the width of the villi, the depth of the crypts, and the width of the crypts were examined morphometrically. An UltraVision Quanto Detection System HRP Polymer (ThermoFisher, USA) was used as a detection system. In group I, there were pronounced inflammation in the form of lymphoplasmacytic infiltration, shortening and thinning of intestinal villi. The number of CD3+ cells, which were located mainly in the center of the upper part of the villi, increased significantly. Bcl-2 expression decreased. In group II, shortening and thinning of intestinal villi progressed. Paneth's cells disappeared. There was a significant decrease in CD3 + and almost complete absence of Bcl-2+ cells ($p \leq 0.05$). Thus, in this work, the dynamics of the expression of pro-inflammatory and anti-apoptotic markers and their relationship with a complex of morphological changes in the wall of the small intestine of rats during the modeling of acute enteric failure have been established.

**KEYWORDS** — enteral insufficiency syndrome, markers of apoptosis, modeling of intestinal obstruction, apoptosis of enterocytes, CD3, Bcl-2.

## INTRODUCTION

The most common cause of acute enteral failure (AEF) in adults is acute intestinal obstruction (mechanical or paralytic ileus). The incidence of acute intestinal obstruction in Russia is about 5 cases per 100 thousand people. Mortality in this pathology remains high and, according to different authors, ranges from 5.1 to 8.4%. According to American authors, intestinal obstruction accounts for approximately 15% of all visits to the emergency department for acute abdominal pain [1, 2]. Many studies have been published on AEF, however, its pathogenesis remains a mystery. The etiology of AEF is multifactorial. Mechanisms involved in AEF include inhibitory sympathetic effects; release of hormones, neurotransmitters and other mediators; inflammatory response; and the effects of analgesics. Endogenous opiates that are released after surgery, according to some authors, may cause AEF. One of the most common causes of AEF is peritonitis. In this case, a superadded infection gradually occurs. Bacterial lipopolysaccharide causes AEF, initiating an inflammatory response in the layers of intestinal smooth muscles. The most dangerous, among others, in the pathogenesis of AEF, is the inhibition of the barrier function of enterocytes, which leads to bacterial translocation into the portal tract and generalization of the infection. With the use of immunohistochemical methods, the possibilities of understanding the pathogenesis of AEF have significantly expanded. A significant role in the development of inflammation belongs to various phenotypes of T-cells CD3, a multifunctional protein marker of T-lymphocytes, responsible for signal transmission after antigens recognition by the T-cell receptor. CD3 regulates the proliferation of T-lymphocytes, and the release of cytokines. CD3 is often considered as a quantitative marker of immune damage to the intestinal wall. In addition to this, a change in the expression of the antiapoptotic protein Bcl-2 makes it possible to assess the state of enterocytes [3, 4].

*Purpose of the study* is to study the diagnostic significance of markers CD3 and Bcl-2 for determining the stage of AEF under experimental conditions.

## MATERIALS AND METHODS

The experimental part of the work was carried out in certified laboratories. The experiment was carried out in compliance with Directive 2010/63 / EU. There is a conclusion of the Ethics Committee (Minutes No. 3 dated 31.10.2011). As a model, we used a technique developed by us for the experimental reconstruction of AEF [5]. The study was carried out on 36 male Wistar rats weighing 250 ± 30 g, comparable in age. The animals were orally administered Loperamide (LSR-004065/09) 2 times a day at a dose of 0.09 mg/day

per kilogram of body weight with an interval between ingestions of 12 hours, for 5 days. Animals under anesthesia were withdrawn from the experiment after 72 and 120 hours with the formation of compensated (group I n = 12) and decompensated (group II n = 12) AEF. The results were compared with a control group of intact animals (n = 12). Small intestine samples were taken for morphometric and immunohistochemical studies. Paraffin blocks were prepared according to the standard procedure. Rabbit antibodies to Bcl-2, clone SP66 (Roche, Switzerland) were diluted 1: 200 in an antibody diluent, rabbit antibodies to CD3 (Prime-BioMed, Russia) were diluted 1: 200. An UltraVision Quanto Detection System HRP Polymer (ThermoFisher, USA) was used as a detection system. The obtained samples were examined using an Olympus BX53 microscope (Olympus, Japan) with a set of objectives UPlanFL N 4x/0.13, UPlanFL N 10x/0.30, and UPlanFL N 40x/0.75. Photos were taken with an Infinity 2 camera (Lumenera, Canada) at 40x and 100x magnifications. The following parameters were studied: length of the villi (VL), width of the villi (VW), depth of the crypts (CD), width of the crypts (CW). Optical density and expression area were determined for each marker. The relative area of expression (%) was calculated as the ratio of the area occupied by immunopositive cells to the total area of cells in the field of view and expressed as a percentage. The optical density of expression was measured in arbitrary units. To assess the differences between the groups of subjects, a parametric unpaired t-test was used. The level of 0.05 was chosen as the critical threshold of significance. To establish a correlation, the Pearson pair correlation coefficient was calculated for nominal variables. To describe the data, the mean value (m) was used, indicating the standard deviation (sd). Statistical processing of the data obtained during the experiment was carried out using the statistical software packages Microsoft Excel 2010 and STATISTIKA 6.0.

## RESULTS AND DISCUSSION

In the control group, a thick mucosa was morphologically visualized, practically not disturbed from the side of the lumen. Wide and long villi. VL averaged 365.3 ± 4.3 μm, the width of the villi, VW, was 65.3 ± 0.9 μm, the CD — 76.3 ± 0.6 μm, the width of the crypts, CW, was 31.3 ± 0.4 μm. In the control group, cells, different from the background, stained with Bcl-2 were noted.

In the morphological study in group I, the histological picture was characterized by pronounced inflammation in the stroma of the villi, there was a pronounced lymphoplasmacytic infiltration without admixture of neutrophils with a small number of eosinophils and Paneth cells. Desquamation of the epithelium took place, however, no areas of exposure of the deep mucosa were observed. No cell lysis was observed. With morphometry, VL averaged 205.3 ± 6.3 μm, VW — 55.3 ± 1.3 μm, CD — 63.3 ± 0.9 μm, CW — 26.3 ± 0.5 μm ($p \leq 0.05$). The average number of boluses on the 2nd day was 1.1, and then there was no defecation. Immunohistochemical changes in the first group: there was a large number of CD3-positive (T-cells), which are located mainly in the upper part of the villi (in the center); in addition, Bcl-2+ cells are also found in the same zones. In the center of the intestinal villi, an inflammatory infiltrate with a predominance of CD3+ T lymphocytes is observed; T cells are also localized in the upper part of the villi (Fig. 1).

In the group II, the picture was characterized by thinning of the mucous membrane of the small intestine, lysis of cells in the upper part of the villi, while the base of the villi was enlarged due to the abundance of lymphoid cells. Paneth's cells were missing. Morphometric analysis revealed significant changes in the sizes of villi and crypts — VL 145.5 ± 5.2 μm, VW — 42.3 ± 0.8 μm, CD — 53.3 ± 0.9 μm, CW — 22.2 ± 0 .5 μm ($p \leq 0.05$). Defecation was absent at all observation periods. A decrease in the number of T-cells (CD3) was noted for due to the lysis of the villi, only their lower third was preserved, where the cells are located. There was practically no Bcl-2 expression in group II (Fig. 2).

Thus, the dynamics of the expression of markers and their relationship with the complex of morphological changes in the wall of the small intestine of rats have been established during the modeling of AEF. In the group I, pronounced inflammation in the form of lymphoplasmacytic infiltration was noted. In the epithelium of the villi, there are single dystrophically altered cells that look like large vacuoles. In single fields of view, desquamation of the epithelium of enterocytes in the apical part is noted. The number of CD3+ cells, which were located mainly in the center of the upper part of the villi, increased significantly. The expression of Bcl-2 decreased slightly in comparison with the control group ($p \geq 0.05$). In the group II, shortening and thinning of intestinal villi progressed. The thickness of the intestinal wall decreased. Paneth's cells disappeared. Massive desquamation of epithelial cells was noted both in the area of the upper parts and lateral walls with exposure of the connective tissue base. There was a significant decrease in CD3+ and almost complete absence of Bcl-2+ cells ($p \geq 0.05$).

## CONCLUSION

Based on the studies carried out, it can be concluded that CD3 and Bcl-2 markers are diagnostically

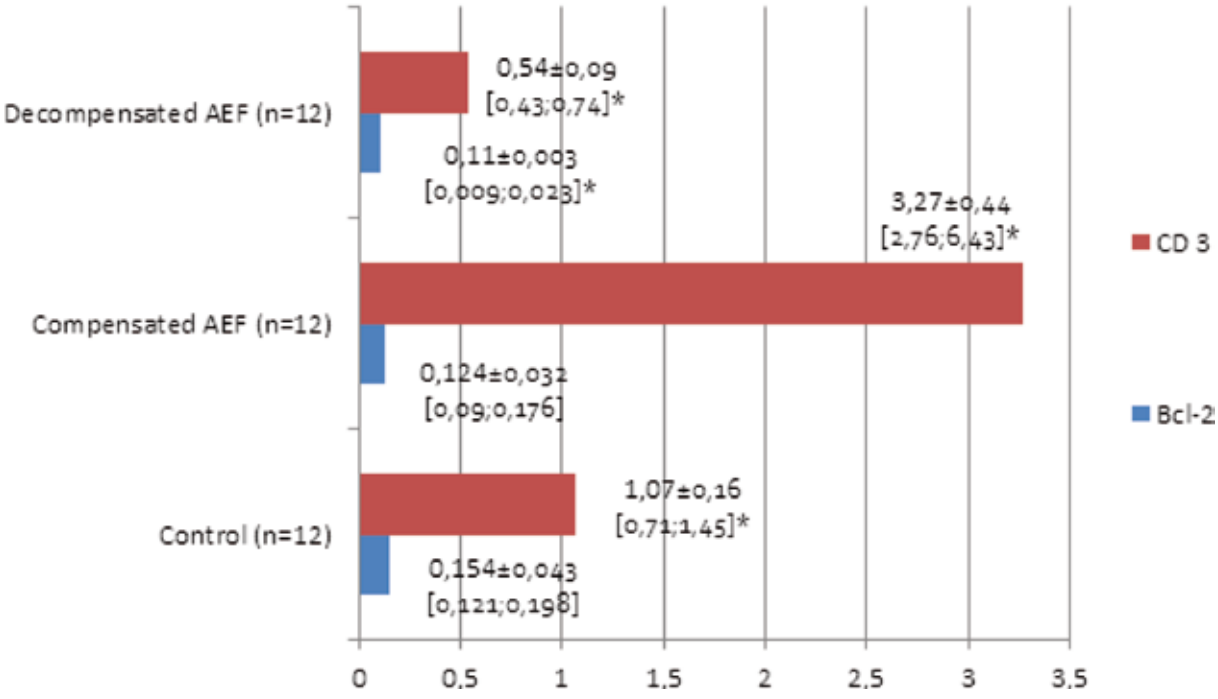


***Fig.1.*** *Optical density of enterocytes immunopositive to Bcl-2 and CD3 in the study groups (s.u.; Me [min; max]; M ± sd)*
** — reliability of changes in values at p≤0.05 in relation to the previous study*

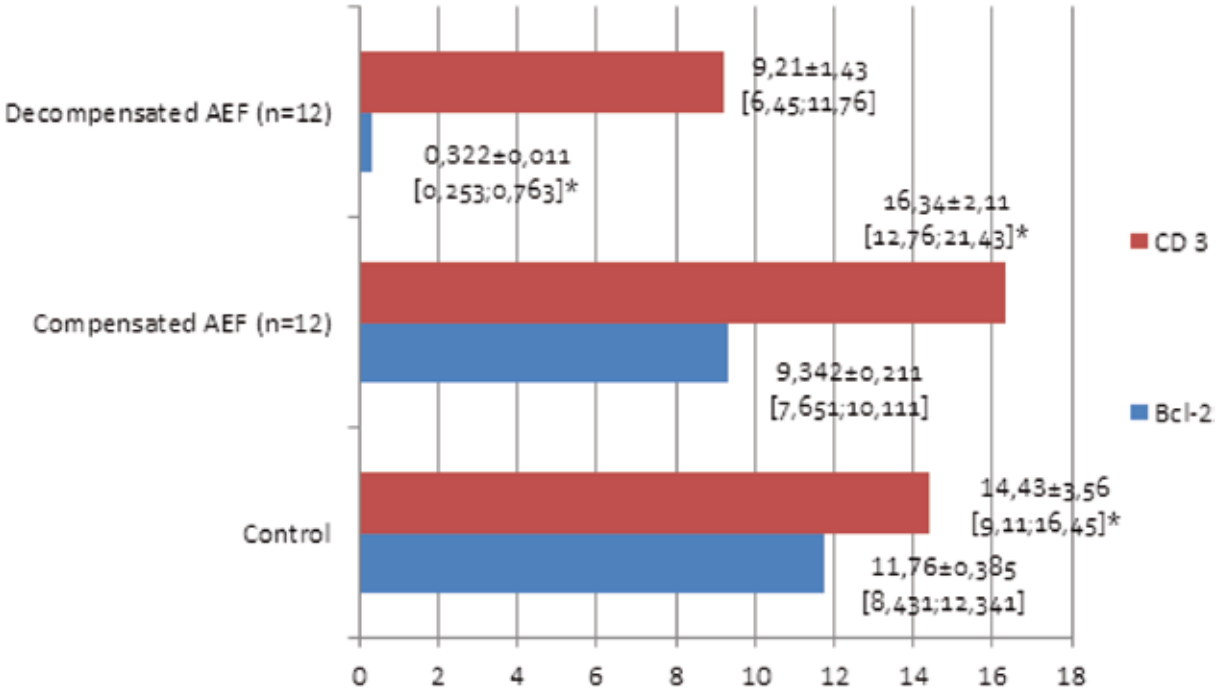


***Fig. 2.*** *Optical area of expression of enterocytes immunopositive to Bcl-2 and CD3 in the study groups (%; Me [min; max]; M ± sd)*
** — reliability of changes in values at p≤0.05 in relation to the previous study*

significant for determining compensated and decompensated stages of AEF. The model used can be applied in experimental surgery to develop and test new treatment methods.

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# A TISSUE REACTION TO COMBINED HERNIA PROSTHESIS AT DIFFERENT POSTOPERATIVE PERIODS

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**ABSTRACT** — Our experimental study shows a morphological response of the anterior abdominal wall to implantation of a combined (bovine pericardium graft and polyester mesh) hernia prosthesis. For this purpose, fifteen chinchillas were operated on. A combined prosthesis was implanted intra-abdominally, where the pericardium graft adjoined the internal organs with the smooth side, while the synthetic material was facing the peritoneum. Morphological and morphometric assessment of the tissue in the area of implantation of the prosthesis was carried out. Morphology of tissues samples was evaluated in 2 weeks, in 1 month and in 2 months after surgery. Our study revealed that the combined prosthesis implanted in the anterior abdominal wall causes an acute inflammatory response, which progresses within a month after the surgery. However, the signs of inflammation reduced at the end of the second month of the experiment. A new connective tissue grew actively into the implant from the side of the polyester mesh. The pericardium graft adjacent to the abdominal cavity with the smooth side did not cause formation of connective tissue. Thus, the combined hernia prosthesis has protected the abdominal cavity from adhesions in the postoperative period.

**KEYWORDS** — pericardium, polyester mesh, morphological reaction.

## RELEVANCE

Currently, hernias of the anterior abdominal wall occupy one of the first places in the field of surgical diseases [1, 2]. Application of synthetic materials for reconstruction of the anterior abdominal wall made a revolution in hernia repair and is considered the gold standard for treatment of patients with central hernias. About 1 million implants of mesh prostheses are produced annually in the world. In some countries, more than 90% of all surgical interventions are performed using synthetic mesh prostheses [3, 4].

There are many studies comparing hernioplasty techniques. It is believed that the most effective method is intra-abdominal surgery [5]. An intra-abdominal implantation of hernia prosthesis is supposed to minimize surgical trauma, but to date there is no unanimous recommendation on the best prosthesis for intra-abdominal hernia repairs [6]. Synthetic prostheses with anti-adhesive coating are conventionally used, but they are expensive and not manufactured in Russia.

The use of xenopericardial animals (pigs, calves) for prostheses of heart valves and blood vessels has long been a practice in cardiovascular surgery [7]. Studies have been conducted in which hernioplasty was performed using a xenopericardial graft. This biological material has several advantages over traditional mesh implants.

In addition, there are studies in which it is shown that polyester mesh prostheses cause a less pronounced inflammatory response than polypropylene ones. However, polyester nets, when sewn into the abdominal wall, undergo sacking [8]. The combination of such a mesh with a xenopericardial graft allows us to solve this problem.

*The purpose* of this experimental study was to study the patterns of morphological reaction of the tissues of the anterior abdominal wall to implantation of a combined (xenopericardial graft and polyester mesh) hernia prosthesis.

## MATERIALS AND METHODS

The experiment was conducted on fifteen chinchilla rabbits weighing up to 3.5 kg. The studies were carried out in accordance with the requirements of the "European Convention for the Protection of Vertebrate Animals, used for experiments or other scientific purposes "(Strasbourg, 1986). Permission was obtained from the local Ethics Committee of the Medical Institute of Penza State University.

The combined prosthesis was made from a bovine pericardium sheet ("Kardioplant", Penza, Russia) and a polyesther mesh (Covidien, Sofradim, France). The prosthesis was implanted intraabdominally. In this case, the xenopericardial graft adjoined the internal organs with the smooth side, and the synthetic material was facing the peritoneum. Animals were removed from the experiment after 2 weeks, 1 month and 2 months after the start of the study. Morphological and

morphometric assessment of the state of tissue in the area of implantation was conducted.

## RESULTS

The combined hernia prosthesis when implanted in the anterior abdominal wall causes a rather pronounced inflammatory reaction which amplifies within a month after the operation. By the second month of the experiment, signs of inflammation were reduced. In this case, new connective tissue grows actively into the implant from the side of the polyester mesh. The xenopericardial graft is anchored to the abdominal wall with the smooth side and does not promote spontaneous endothelialization, thus protecting the abdominal cavity from adhesions in the postoperative period.

## DISCUSSION

The study showed that the combination of a xenopericardial graft and a polyester mesh as a single hernia prosthesis leads to the development of a sufficiently pronounced inflammatory response in the tissues of the abdominal wall.

In the early stages — 2 weeks after surgery, inflammatory infiltration is mainly detected in the area of the polyester network, later lymphocytes and neutrophilic leukocytes spread into the thickness of the xenopericardial graft. A month after the start of the experiment, inflammation in the area of operation reaches its maximum intensity, after that it gradually decreases.

The formation of connective tissue around the implantation is very active. Initially, a large number of fibroblasts and, accordingly, connective tissue fibers are formed at the border of the intrinsic tissues of the abdominal wall and the polyester mesh.

Then the fibers and capillaries of the granulation tissue grow into the mesh cells, approaching the xenopericardial graft. The development of the inflammatory reaction and the growth of connective tissue around the combined prosthesis has a number of features compared to separately used mesh implants [7, 8].

At the final stage of the experiment (after 2 months), the preparations show a fairly dense connective tissue fused with a polyester mesh. In this case, the formation of connective tissue from the xenopericardial graft does not occur. In this way, the use of combined prostheses can help in solving the problem of adhesion formation after hernioplasty, which is repeatedly described in the literature [9, 10].

## CONCLUSION

The use of our combined prosthetic option for implantation into the abdominal wall enables to avoid a number of complications that often arise with other types of prostheses.

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# LAPAROSCOPIC AND ROBOTIC-ASSISTED SURGERY FOR COMPLICATED DIVERTICULITIS

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**ABSTRACT** — The article presents the results of a longtime experience with the effects of perioperative intestinal decontamination in patients with complicated diverticulitis on the incidence of postoperative complications following delayed minimally invasive sigmoid colectomy and colorectal anastomosis creation.
PURPOSE. This work aims to evaluate the prophylactic potential of selective perioperative intestinal decontamination in patients with sigmoid diverticulitis due to perforation of the inflamed diverticula, as a form of diverticular disease; to study the incidence of postoperative complications in patients with complicated diverticulitis following laparoscopic and robotic-assisted surgery.
MATERIALS AND METHODS. We studied the short-term postoperative outcomes of 179 patients with sigmoid diverticulitis associated with perforation of the inflamed diverticula using both prospective and retrospective methods. The study participants were divided into two groups: Group A (n=91) (50.8%)), of which 65 patients (36.3%) underwent laparoscopic and 26 patients (14.5%) — robotic-assisted surgery and received intestinal decontamination peri-operatively, and Group B, consisting of 88 patients (49.2%), who underwent laparoscopy but did not receive intestinal decontamination. In all cases, sigmoid colectomy was performed and a primary colorectal anastomosis was formed.
RESULTS. Of 179 patients included in the study, 136 (76%) did not develop postoperative complications. In Group A, 11 (12%) of 91 patients and in Group B, 17 (19.3%) of 88 patients developed postoperative complications specific to the type of surgery undertaken. The most common complications included colorectal anastomotic leakage, adhesive intestinal obstruction, anastomotic bleeding, intra-abdominal hemorrhage and localized peritonitis. A statistical comparison revealed no statistically significant differences between the groups studied. 8 (8.8%) of 91 patients in Group A and in 7 (8.0%) of 88 patients in Group B were diagnosed with extra-abdominal (non-surgical) complications. Thus, the total postoperative complication rate was lower in Group A (n=19 (20.9%) than in Group B (n=24 (27.3%)) ($X^2$=1.002, p=0.316). Colorectal anastomotic leakage rates were lower in Group A (n=1 (1.1.%)) than in Group B (n=4 (4.6%)) (F=0.205, p>0.05). Wound infection rates in Group A were 6.6% (n=6) patients and in Group B – 11.3% (n=10) patients ($X^{2*}$=6.483, p=0.01). No deaths were reported.
CONCLUSION. Selective intestinal decontamination combined with oral decontaminating solutions has been shown to reduce the occurrence of colorectal anastomotic leaks, wound infection, surgical and general postoperative complications.

**KEYWORDS** — selective intestinal decontamination (SID), diverticular disease, robotic-assisted and laparoscopic sigmoid colon resection, colorectal anastomotic leakage (AL), postoperative complications.

## INTRODUCTION

Diverticular disease is a prevalent inflammatory disorder that affects the digestive tract. The prevalence of diverticular disease is estimated to be as low as 2% in those 30 years or younger, 5% in those 40 years of age and it is estimated to be as high as 70% by 80 years of age [1, 2]. Although 80% of patients tend to have uncomplicated presentations, 20% develop various types of diverticulitis [3, 4].

The current Hansen and Stock classification of colonic diverticular disease is used as the standard for clinical practice in the guidelines for the treatment of diverticular disease in Germany [5]. Complicated diverticulitis associated with perforation of the intestinal wall and pericolic abscesses have been a subject of increasing interest for surgeons. Patients with complicated diverticulitis often require numerous hospitalizations and/or surgery [6, 13].

Approximately 15% of patients develop perforated diverticulitis associated with pericolic abscesses measuring 5 cm in size or larger. In such cases, ultrasound or CT imaging can be used to guide abscess drainage [7, 8]. Patients with complicated diverticulitis often require sigmoid resection down to the rectosigmoid junction to avoid diverticulitis relapses and complications. However, potential risks of bowel resection are mainly those of any major complications including anastomotic leakage (AL), which is the most common cause of prolonged hospital stays and may even lead to death in 5.0–20.0% of cases [9, 12].

Recent studies with *Pseudomonas aeruginosa* and *Enterococcus faecalis* have shown that various concentrations of these bacteria, which are capable of activating intestinal tissue matrix metalloproteinase-9 (MMP9) and degrading collagen, lead to the development of AL [10]. The negative impacts of the gut

*Pseudomonas aeruginosa* and *Enterococcus faecalis* can be significantly reduced with the use of perioperative selective decontamination [11].

*The purpose of our study* was to evaluate the prophylactic potential of perioperative SID in patients with sigmoid diverticulitis associated with perforation of the inflamed diverticula and to study the occurrence of general and specific to the type of surgery undertaken postoperative complications in patients following laparoscopic colorectal resection.

## MATERIALS AND METHODS

Between January 2015 and October 2020, in the Department of General, Oncologic and Thoracic Surgery of Sana Klinikum Hof GmBH, University Hospital of Erlangen, Friedrich-Alexander University of Erlangen-Nürnberg (Bavaria, Germany) 191 patients were operated on for acute complicated diverticulitis (Type IIa, IIb) using conventional and robotic-assisted laparoscopy. The patients were eligible for the study if they had been diagnosed of having sigmoid diverticulitis, provided informed consent for surgery and anesthesia, and were good candidates for a primary anastomosis. The exclusion criteria included high risk of anesthesia-related complications (ASA class IV), immunosuppression, severe diabetes mellitus, and end-stage renal disease (ESRD).

A total of 179 patients who consented were enrolled in the study. 91 patients who received oral decontamination perioperatively were attributed to Group A, while 88 patients, comprising Group B, underwent major surgery without receiving perioperative oral decontamination.

The conservative treatment involving administration of intravenous antibiotics and analgesics as well as high-calorie parenteral nutrition solutions was followed by sigmoid resection, of which 153 resections (65 (71.4%) in Group A) and 88 (100%) in Group B) were performed laparoscopically, and 26 ((28.6%) in Group A) resections — using a robotic-assisted technique.

Primary colorectal anastomoses were formed using a circular stapling instrument ~10–12 cm above the anal sphincter. The mean age of patients in Group A was 58±12.5 year, while in Group B — 55.6±13.2 (Student's t test =1.368, p=0.086). Females accounted for 50.5% (n=46) of the total sample size in Group A, and 42 (47.7%) in Group B. The study population comprised 45 (49.5%) males in Group A and 46 (52.3%) males in Group B, respectively. There were no statistically significant gender differences in the study groups ($X^2$=0.142, p=0.705).

The majority of patients in the study groups were classified as ASA class II ($X^2$=0.05, p=0.82) or ASA class III ($X^2$=0.01, p=0.903). No statistically significant differences between ASA III and ASA II patients in Group A and Group B were found. Baseline CRP level (110±75.4 mg/L) was statistically lower in Group B (Mann-Whitney U test =2.733, p=0.006) than in Group A (130.7±59.2 mg/L). For WBC counts, we found no statistically significant differences in the study groups (Student's t test =2.005, p=0.88).

In all cases, the diagnosis of complicated diverticulitis was confirmed with CT imaging with intravenous contrast. If a pericolic abscess measuring > 5 cm in size was available, CT imaging was used to guide abscess drainage. Pericolic abscesses were identified in 35 (38.5%) of 91 patients in Group A and in 21 (23.7%) of 88 patients in Group B ($X^2$=5.05, p=0.02). In Group A, external drainage prior to surgical resection was performed in 7/35 (7.7%) of 35 cases, in Group B – in 3/21(3.4%) of 21 cases. No statistically significant differences in the frequency of use of this treatment approach were found in the study groups (F=0.72, p>0.05).

In Group A, patients received intestinal decontamination in the conventional manner: one day before surgery and after mechanical bowel preparation the patients received the first dose of neomycin (1 g) and 800 mg of metronidazole at 7 and 11 pm. The patients received a second dose of neomycin (500 mg) at 6 am on the day of surgery. Postoperatively, the patients received metronidazole at a dose of 400 mg orally twice a day for 5 days.

## RESULTS AND DISCUSSION

The general and postoperative length of hospital stay was significantly shorter in Group A and amounted to 13.9±4.1, while in Group B — 16.1±6.1 days (Student's t test =-2.721, p=0.003). Postoperative hospital stay for Group A patients was 8.1±3.6 and for Group B patients — 9.3+5.5 days (Student's t test = -1.883, p=0.036). The duration of laparoscopic surgery in Group A was significantly shorter (173±45.6 minutes) than in Group B (190.1±50.1 minutes) (*z=2.1758, p=0.02926).

Two (1.1%) of 179 patients developed intraoperative complications. Ureteral trauma occurred in one patient in Group A during the surgery. The repair of ureteral trauma involved creating a reanastomosis between the bladder and the proximal ureter. Moreover, one patient in Group B developed bleeding from the staple line (anastomosis) which was arrested with endoscopic clipping.

The number of conversions from laparoscopic surgery to an open approach was greater in Group B —

10 (11.4%) of 88 patients than in Group A — 5 (5.5%) of 91 patients ($X^2$ with Yates's correction =1.316, p=0.252). The reasons for conversion to an open approach included large pelvic abscesses (3 cases) which for some technical reasons had not been drained prior to surgery; internal fistulas (5 cases) which required additional resection of the urinary bladder, uterus or the small intestine; extensive peritoneal adhesions arising due to inflammation (7 cases) whose separation with a minimally invasive approach was impossible.

In Group A, anastomotic leakage occurred in 1 patient (1.1%) and it was treated with re-laparoscopy, pelvic lavage, formation of a double-barrel ileostoma and placement of a transrectal endoscopic vacuum-assisted system. On day 21 postoperatively, the patient had resolution of the anastomotic defect which had been covered with granulation tissue. The protective double barrel ileostoma was closed 42 days following the initial surgery. In Group B, AL occurred in 4 patients (4.55%). In 3 patients AL was treated with a minimally invasive approach, which involved laparoscopic lavage, abdominal drainage, formation of a distal ileostoma and placement of a transrectal endoscopic vacuum-assisted system.

One patient underwent Hartman's procedure by laparotomy due to colorectal anastomotic failure related to a large size of the AL, intestinal ischemia and severe fecal peritonitis. A comprehensive intensive treatment program helped to stabilize the patient and plan rehabilitation and follow-up for him. The period of time from the patient's discharge from hospital to the closure of the single-barrel colostoma was 6 months. The recovery period was uneventful.

An overview of general and specific to the type of surgery undertaken postoperative complications which occurred in the study groups is presented in Table 1.

## CONCLUSION

Our findings show that SID holds great potential as a treatment approach to patients with diverticulitis associated with perforation of the inflamed diverticula (Type IIa, IIb). It decreases not only the number of postoperative wound infections but also general postoperative complications, including colorectal AL. The study provides evidence of the beneficial effects of intestinal decontamination in preventing complications following laparoscopic or robotic-assisted colorectal resections which are commonly undertaken in complicated diverticulitis.

### *Conflict of Interest*

The authors declare no conflicts of interest.

### *Author Contributions*

Concept and design of the study — O.F. Vorontsov, C. Graeb;

Data collection and processing — O.F. Vorontsov, V.V. Tolochyk, A.V. Kitaeva;

Text writing — O.F. Vorontsov;

Editing — I.V. Mikhin, C. Graeb

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***Table 1.*** *Postoperative complications in the study groups*

| Complication | Study groups | | Statistically significant difference |
|---|---|---|---|
| | A (n=91) | B (n=88) | |
| Extra-abdominal | 8 (8.79%) | 7 (7.95%) | $X^{2*}$=0.004, p=0.945 |
| Wound infection | 6 (6.6%) | 10 (11.3%) | $X^{2*}$=6.483, p=0.01 |
| Intra-abdominal complications | 5 (5.49%) | 7 (7.95%) | $X^{2*}$=0.649, p=0.420 |
| Adhesive intestinal obstruction | 1 (1.1%) | 1 (1.14%) | F=1, p>0.05 |
| Peritonitis | 2 (2.2%) | 0 (0%) | F=0.497, p>0.05 |
| Anastomotic bleeding | 0 (0%) | 1 (1.14%) | F=0.491, p>0.05 |
| Intra-abdominal hemorrhage | 1 (1.1%) | 1 (1.14%) | F=1, p>0.05 |
| Anastomotic leak | 1 (1.1%) | 4 (4.55%) | F=0.205, p>0.05 |
| Total | 19 (20.88%) | 24 (27.27%) | $X^2$=1.002, p=0.316 |

*$X^2$ — the chi-square test, $X^{2*}$ — the chi-square test with Yates's correction, F — Fisher's exact test.*

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# FUNCTION AND COMPLICATIONS AFTER ENDOPROSTHETIC REPLACEMENT OF KNEE BONE-FORMING TUMORS EXPOSED TO RADIOTHERAPY AND MULTIAGENT CHEMOTHERAPY

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**ABSTRACT** — We discuss analysis of function outcome and complications in 65 patients undergoing endoprosthetic knee replacement for osteosarcoma after radiotherapy and multiagent chemotherapy. Specifically, we found that multiagent chemotherapy caused a periprosthetic infection in 7.7% of cases. Major complications of radiotherapy included periprosthetic infection (27.3%), bone fracture at the site of endoprosthesis stem implantation (27.3%), aseptic loosening of stem (18.2%) and in 9.1% of patients a post-radiation skin ulcer was observed. Strategies for eliminating complications of periprosthetic infection included: removal of the endoprosthesis, installation of a metal-cement spacer followed by repeated joint endoprosthesis replacement. Then metal osteosynthesis was performed with cover plates and cable grip in case of bone fractures at the site of endoprosthesis stem implantation. A revision knee replacement surgery was performed in case of aseptic loosening of stem; removal of ulcer, removal of necrotic tissue, wound revision, and wound closure by means of muscle reposition with the subsequent free skin grafting was carried out during removal of postradiation ulcer. Hip amputation or disarticulation of the thigh was carried out in case of recurrence; multiagent chemotherapy with metastasioctomy was performed in case of metastases in lung.

**KEYWORDS** — knee joint, endoprosthetic replacement, chemotherapy, radiotherapy, complications, methods of eliminating complications.

## INTRODUCTION

The review of literature confirms that a combined treatment is used in cases of osteosarcoma of high grade. It includes neoadjuvant chemotherapy (CT) cycle, definitive surgery, adjuvant CT cycle. It is also recommended to use modular endoprosthesis replacement systems, which considered most relevant and advanced. Furthermore, they meet the basic requirements for reconstruction of bone defects in cases of bone sarcomas [1, 2, 4]. A surgical treatment alone for osteogenic sarcoma is impractical, as within 1.5 years after surgery 80–90% of patients are diagnosed with metastases in lungs and local recurrences. CT as part of multimodal treatment significantly improves the 5-year survival in patients with osteogenic sarcoma with a localized process (20% to 60%). The advantages of preoperative CT include the ability to assess the in vivo activity of chemotherapeutic preparations and to facilitate the surgery conducting [5]. Usually 3–4 cycles of preoperative CT are followed by the endoprosthetic knee replacement. After tumor removal, the degree of tumor necrosis after preoperative CT is determined, which is a reliable independent prognostic factor. With a positive response of the tumor to the treatment (tumor necrosis of 90% or more) there is a high probability of recurrence-free and overall survival of patients [1]. Regarding patients with failure response after preoperative CT, as a rule, postoperative CT does not lead to improved survival [2, 4]. Radiotherapy (RT) has been used successfully in the treatment of malignancies of various localizations. The majority of patients with bony spread suffer from pain syndrome and in whom RT reduces or completely cures pain [12]. According to the literature, due to radiation, a partial effect can be achieved in 60–80% of cases, and Total Pain Relief can be achieved in 15–40% of patients [6]. One of the possible ways to increase the effectiveness of therapy for patients with metastatic lesions of skeletal system may be an external-beam RT in combination with the injection of bisphosphonates. During radiotherapy conducting, in most cases, soft tissues, bone tissue and visceral organs located in the immediate vicinity of the tumor fall into the radiation area, which leads to their destruction [8]. Late radioreactions develop in several years after treatment and consist of dysfunction of osteoblasts, osteotabes and replacement of bone marrow by connective tissue, pathological bone fragility, trophism disorders, development of osteonecrosis and osteomyelitis [3, 11]. The above complications are characterized by the term "radiation osteitis". Factors contributing to the development of post-radiation pathological fractures include

a large dose of radiation, high beam energy and the presence of a history of osteoporosis [11]. When using RT in the combined therapy of malignant neoplasms, the typical complications include radiation damage to the integumentary tissues (fibrosis, ulcer in the area of radiation fields, radiation plexopathy, secondary lymphostasis of the upper and lower extremities, radionecrosis (osteoporosis, radiation osteomyelitis) of bones, intrapelvic radiation fibrosis, radiation damage to the intestines, urinary bladder, esophagus, heart, etc. [3, 11] It should be noted that increasing effectiveness of combined treatment of malignant neoplasms, including radiation therapy extends life expectancy of the patients, and therefore detection of radiation damage to bones has become more common, although its development requires a long period of time [9]. In most cases, correction of this pathology requires a surgical treatment due to ineffectiveness of conservative measures. In cases where the diagnosis of radiation damage to the bone is confirmed, it is necessary to partially or completely remove the affected fragment. In addition, radiation osteoradionecrosis and osteomyelitis are usually accompanied by damage to the surrounding soft tissues with the development of radiation ulcers and fistulas. This requires the use of certain types of corrective skin surgery [10]. This paper expands the understanding of the causes of complications after the use of radiotherapy and multiagent chemotherapy during endoprosthetic knee replacement in case of bone tumors, and provides methods for their elimination.

## PURPOSE

The purpose of our research was to analyze the complications occurring after the use of radiotherapy and multiagent chemotherapy during knee endoprosthesis replacement for bone tumors.

## MATERIALS AND METHODS

During the period from 2009 to 2020, endoprosthetic knee replacement was performed in 65 patients with knee bone-forming tumors. Among the treated patients there were 29 women (44.6%), and 36 men (55.4%). The mean age of patients amounted to 27.6 ± 1.4 years. Patients underwent knee joint endoprosthesis replacement using individual modular oncological endoprostheses produced by Stryker and W.Link companies and individual oncological endoprostheses produced by Inmed and Beznoska companies. The use of a modular system simplifies the performance of both the endoprosthesis replacement itself and subsequent revision surgery, increasing the biological reserve of bones. The possibility of manufacturing individual components of the endoprosthesis for revision surgery, with the development of instability allows, without removing the entire endoprosthesis, to replace a part of it. Table 1 presents the histological forms of the tumor and the number of patients who underwent chemoradiation treatment before knee joint endoprosthesis replacement.

In case of osteogenic sarcoma (giant-cell sarcoma of bone, fibrosarcoma of bone, malignant fibro histiocytoma of the bone, Ewing sarcoma, metastatic tumor), depending on the size of the primary site and degree of tumor extension it was decided by a team of surgeon-oncologist-orthopedist-chemotherapist on the feasibility of chemotherapeutical treatment. Chemotherapy in some cases can reduce primary tumor size and metastases, promote its delimitation by a pseudocapsule that allows to transfer a tumor to a resectable condition. 39 patients underwent courses of neoadjuvant and adjuvant multiagent chemotherapy according to treatment protocols of these nosological entities, of which 26 patients had osteogenic sarcoma, 5 of them had giant cell sarcoma of bone, 4 of them had fibrosarcoma of bone, 1 of them had malignant fibro histiocytoma of bone, and 1 of them had metastatic tumor (metastasis of kidney cancer). In case of osteogenic sarcoma (giant cell sarcoma of bone, fibrosarcoma of bone, malignant fibro histiocytoma of bone), we introduced the following regimens of chemotherapy: AP regimen: doxorubicin: 90 mg/m$^2$ intravenous, 96-hour infusion; cisplatin 120 mg/m$^2$ intravenous infusion on the 1$^{st}$ day every 4 weeks of the 4$^{th}$ cycle, regimen I (used at low efficiency after 2 cycles of AR): ifosfamide (with uremitexan) 2000 mg/m$^2$ intravenous on the 1$^{st}$–7$^{th}$ day of the 2$^{nd}$ cycle. During postoperative period we used the following regimens: if the tumor necrosis was more than 90%: doxorubicin: 25 mg/m$^2$ on the 1$^{st}$–3$^{rd}$ day intravenous as a 72-hour continuous infusion; ifosfamide: 2500 mg/m$^2$ on the 1$^{st}$–4$^{th}$ day intravenous with uremitexan, if the tumor necrosis amounted from 50 to 90% then regimen I was used: ifosfamide: 2 g/m$^2$ (with uremitexan) intravenous infusion on the 1$^{st}$–7$^{th}$ day, of the 2$^{nd}$ cycle with an interval of 3 weeks, in 3 weeks — MTX: methotrexate: 12 g/m$^2$ intravenous infusion with leucovorin, 4 injections with an interval of 14 days for young patients, after 3 weeks AI regimen was introduced: doxorubicin: 25 mg/m$^2$ on the 1$^{st}$–3$^{rd}$ day intravenous as a 72-hour continuous infusion, ifosfamide: 2500 mg/m$^2$ on the 1$^{st}$–4$^{th}$ day intravenous with uremitexane. All three regimens were repeated 3 times. If the tumor necrosis was less than 50%, the same regimens were used three times, but with the replacement of the AI regimen with the GemTax regimen: gemcitabine 900 mg/m$^2$ on the 1$^{st}$, 8$^{th}$ day as a 90-minute infusion, docetaxel: 100 mg/m$^2$ on the 8$^{th}$ day. The

***Table 1.*** *Histological Forms of the Tumor and the Number of Patients Who Underwent Chemoradiation Treatment before Endoprothetic Knee Replacement*

| Nosological entity of the tumor | Number of cases, % | Chemoradiation, number of cases, % |
|---|---|---|
| Giant cell tumor of bone | 26 (40%) | 7 (26,9%) patients underwent radiotherapy |
| Osteogenic sarcoma | 26 (40%) | 26 (100%) patients underwent multiagent chemotherapy |
| Giant-cell sarcoma of bone | 5 (7.7%) | 5 (100%) patients underwent multiagent chemotherapy, of them 3 (60%) patients additionally underwent radiotherapy |
| Fibrosarcoma of bone | 4 (6.2%) | 4 (100%) patients underwent multiagent chemotherapy |
| Malignant fibro histiocytoma of bone | 2 (3.1%) | 2 (100%) patients underwent multiagent chemotherapy |
| Ewing sarcoma | 1 (1.5%) | 1 (100%) patient underwent multiagent chemotherapy and radiotherapy |
| Metastatic tumor | 1 (1.5%) | 1 (100%) patient underwent multiagent chemotherapy and radiotherapy |
| Total amount | 65 (100%) | 39 (60%) patients underwent multiagent chemotherapy, 12 (18,4%) patients underwent radiotherapy, a total number of 51 (78.5%) patients were treated |

duration of treatment amounted to about 12 months. In case of Ewing sarcoma without metastases, we carried out chemotherapy according to the Scandinavian protocol. The treatment regimen included the following cytostatics: doxorubicin, ifosfamide, vincristine, dactinomycin. In the case of metastatic kidney tumor, targeted therapy was performed, which consisted of taking nexavar orally at a dose of 400 mg per day for a long time. In cases of giant cell tumor of bone, we used regimens, which included denosumab (prolia) 60 mg on the 1$^{st}$, 8$^{th}$, and 15$^{th}$ day, followed by organ-sparing surgery in the form of resection of bone with the tumor and knee joint endoprosthesis replacement. Twelve patients in the preoperative stage underwent a course of external-beam radiotherapy to a total radiation dose of 40 Gy, at a single tumour dose (amounts to 2–2.5 Gy), among which there were 7 patients with giant cell tumor of bone, 3 patients were with malignant giant cell tumor of bone, 1 patient with Ewing sarcoma, and 1 patient with metastatic tumor. Radiotherapy was indicated for treatment in case of these nosological entities due to the fact that these tumors according to experimental-clinical studies are sensitive to radiotherapy. The scope of surgical interference consisted of resection of the articular segment of the bone with an *en block* tumor and replacement of the bone defect with an individual oncological or individual modular oncological endoprosthesis. The functional result of the operated limb was calculated according to the MSTS scale (Musculo-Sceletal Tumor Staging/System/). Quality of life was determined as per EORTIC-QLQ-C30 questionnaire. Patient survival was assessed using the Kaplan-Meier method.

## RESULTS AND DISCUSSION

As a result of knee endoprosthesis replacement conducted for the group (n = 39) of patients, who underwent courses of neoadjuvant and adjuvant multiagent chemotherapy, 16 (41%) patients presented with complications after endoprosthesis replacement: 3 (7.7%) patients presented with periprosthetic infection, 3 (7.7%) patients presented with tumor recurrence, 10 (25.6%) patients presented with metastases in lungs. After multiagent chemotherapy, almost all patients had leukopenia, anemia, thrombocytopenia, which was corrected by replacement therapy (administration of the Zarsio or Filstim drug, transfusion of one-group blood, platelet concentrate or thrombo-concentrate). 8 (20.5%) patients died during treatment due to metastases in lungs. In the group (n = 12) of patients who underwent a course of radiotherapy up to total radiation dose of 40 Gy in the preoperative period, 9 (75%) patients presented with complications after endoprosthesis replacement: 3 (27.3%) patients presented with periprosthetic infection, 3 (27.3%) patients presented with bone fracture at the site of endoprosthesis stem implantation, 2 (18.2%) patients presented with aseptic loosening of stem, 1 (9.1%) patient, having metastatic tumor (metastases of kidney cancer), presented with a post-radiation skin ulcer in the proximal crural region, suture line disruption, fistula, although inoculation from fistula to the microflora was negative. Data on complications patients presented with after chemoradiation during knee joint endoprosthesis replacement in terms of each nosological entity of the tumor are shown in Table 2.

3 patients having giant cell tumor of bone with periprosthetic infection underwent the following: in 2 cases revision of postoperative wound has been conducted, dialysis and a powerful course of antibiotic therapy has been produced, in the 1st case it was a removal of the endoprosthesis, an installation of a metal-cement spacer device followed by repeated joint endoprosthesis replacement. In cases of bone fracture at the site of endoprosthesis stem implantation in 2 cases metallic osteosynthesis was performed with cover

***Table 2.*** *Complications caused by chemoradiation during knee replacement*

| Nosological entity and number of patients | Provided treatment | Complications after treatment (including endoprosthesis replacement) |
|---|---|---|
| Giant cell tumor of bone – 26 patients | 7 patients had radiotherapy | periprosthetic infection – 3 (42,8%), bone fracture at the site of endoprosthesis stem implantation – 2 (28,6%), aseptic loosening of stem – 2 (28,6%) |
| Osteogenic sarcoma – 26 patients | 26 patients had multiagent chemotherapy | Periprosthetic infection – 2 (7,6%), tumor recurrence – 2 (7,6%); metastases – 9 (34,6%) |
| Giant-cell sarcoma of bone – 5 patients | 5 patients had multiagent chemotherapy, 3 of them – radiotherapy | Bone fracture at the site of endoprosthesis stem implantation – 1 (33,3%) |
| Fibrosarcoma of bone – 4 patients | 4 patients had multiagent chemotherapy | Periprosthetic infection – 1 (25%) |
| Malignant fibro histiocytoma of bone – 2 patients | 2 patients underwent multiagent chemotherapy | Metastases – 1 (50%) |
| Ewing sarcoma – 1 patient | This patient underwent multiagent chemotherapy and radiotherapy | Tumor recurrence – 1 (100%) |
| Metastatic tumor (metastases of kidney cancer) – 1 patient | This patient underwent multiagent chemotherapy and radiotherapy | Post-radiation ulcer – 1 (100%) |
| Totally 65 patients | 39 patients underwent multiagent chemotherapy, 12 patients underwent radiotherapy, totally 51 patient underwent treatment | Periprosthetic infection – 6 (11,8%), bone fracture at the site of endoprosthesis stem implantation – 3 (5,9%), aseptic loosening of stem – 2 (3,9%), post-radiation ulcer – 1 (2,0%), tumor recurrence – 3 (5,9%), metastases – 10 (19,6%) |

plates and cable grip. In case of aseptic loosening of stem in 2 cases repeated joint endoprosthesis replacement was performed, in one case replacement was performed of complete endoprosthesis; in the other case only one femoral stem of endoprosthesis was replaced with the longer one. In respect of 2 patients with osteogenic sarcoma of bone with periprosthetic infection, removal of the endoprosthesis, and installation of a metal-cement spacer followed by repeated joint endoprosthetic replacement was performed, in respect of 2 patients with tumor recurrence in the 1st case hip amputation was performed, in the 2nd case hip disarticulation of hip joint was performed. In case of metastases in lungs, cycles of multiagent chemotherapy with metastasectomy were performed. Regarding the patient with a giant cell sarcoma of bone with a bone fracture at the site of endoprosthesis stem implantation, metallic osteosynthesis with periosteal plate and cable grip was performed. Regarding the patient with fibrosarcoma of bone with periprosthetic infection, the endoprosthesis was removed and a metal-cement spacer was installed, followed by repeated joint endoprosthetic replacement. The patient with a malignant fibrous histiocytoma of bone with multiple metastases in lungs received cycles of multiagent chemotherapy. The patient with Ewing sarcoma underwent hip amputation and courses of multiagent chemotherapy due to tumor recurrence. The patient with a metastatic tumor, where post-radiation ulcer complications were observed, underwent removal of the ulcer, removal of necrotic tissue, revision of the knee joint, and closure of the soft tissue defect and skin defect by shifting the medial gastrocnemius followed by free skin grafting. Stages of surgical interference are shown at Fig. 5–7.

The functional result (MSTS scale) of the lower extremity amounted to 88.2% after resection of the distal femur and knee joint endoprosthetic replacement, and functional result (MSTS scale) of the lower extremity amounted to 82.4% after resection of the proximal tibia and knee endoprosthetic replacement. Quality of life after knee joint endoprosthesis replacement (EORTIC-QLQ-C30 questionnaire) increased from 40 points before endoprosthesis replacement, to 80 points after endoprosthetic replacement. The overall three-year survival of patients was 68.2 ± 2.4%, the five-year survival of patients was 51.8 ± 3.2%.

***An case study from our practice:*** Patient G., 62 years-old was admitted to the Institute of Traumatology and Orthopedics (Kyiv, Ukraine), with the following diagnosis: metastasis of kidney cancer to the distal segment of femoral bone, clinical group II. According to her medical case history she underwent nephrectomy in 2018. In 2019 the patient felt pain in her left thigh. The destruction in the distal femur of her thigh was detected at an additional x-ray examination (Fig. 1). Core needle biopsy of a neoplasm of the femoral bone was performed. Pathohistological findings: metastasis of clear cell renal cell cancer. After that the patient received a cycle of radiotherapy for femoral metastasis in the total radiation dose of

40 Gy. Two weeks after radiotherapy a pathological fracture of the femoral bone was observed on the radiograph (Fig. 2). Six weeks later, on August 5, 2020, the patient was operated on: resection of the tumor in distal segment of the left femoral bone followed with knee endoprosthetic replacement using an individual Beznoska endoprosthesis (Fig. 3). In the post-surgery period, a post-radiation skin ulcer in the lower third of the thigh and disruption of the suture line of the postoperative wound were observed (Fig. 4). Inoculation of the postradiation ulcer of the knee joint was carried out, conclusion: no microorganisms were detected. On October 8, 2020, the Patient underwent surgical interference — removal of post-radiation ulcer and necrotic tissue, revision of the knee joint, sanation and transposition of Gastrocnemius medial head to the anterior surface of the knee joint to cover the soft tissue defect (Fig. 5–6). On October 15, 2020, the Patient underwent another surgical interference — free skin grafting of the skin defect of the anterior surface of the left lower leg with an autodermal graft, which was taken from the lateral surface of the left thigh (Fig. 7). In the post-surgery period, complications connected with skin and postoperative wound were not observed, the stitches were removed. The patient was discharged under the supervision of an oncologist.

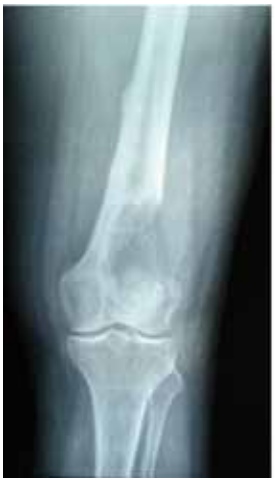

***Fig. 1.*** *Patient G., metastatic lesion of the femoral bone*

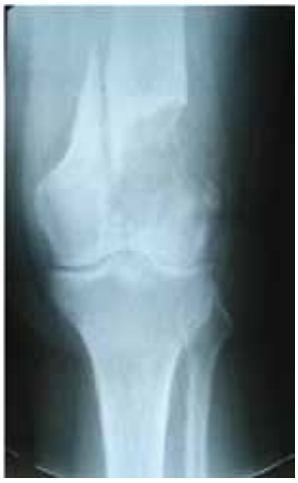

***Fig. 2.*** *Patient G., pathological femur fracture on the background of metastatic lesions after radiotherapy*

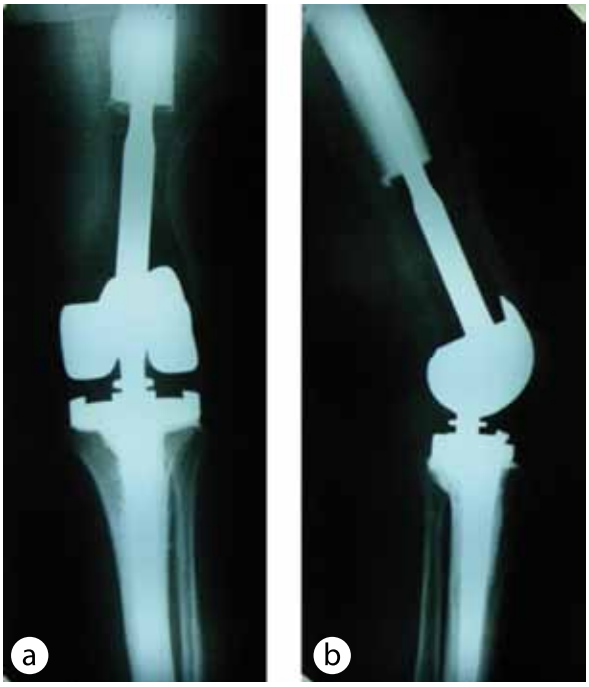


***Fig. 3.*** *Patient G, a — frontal view, b — lateral view — after resection of the distal segment of the femur with a tumor and endoprosthetic replacement using Beznoska endoprosthesis*

## RESULTS AND DISCUSSION

Our outcomes have shown the complications from exposure to multiagent chemotherapy on knee reconstruction. A periprosthetic infection was found in 7.7% of cases. We believe that this complication is related to a decrease in the patient's phylactic power. When using radiotherapy in the preoperative stage, the patients presented with the following complications after endoprosthesis replacement: periprosthetic infection in 27.3% of cases, bone fracture at the site of endoprosthesis stem implantation in 27.3% of cases, aseptic loosening of stem in 18.2% of cases, and post-radiation ulcer in 9.1% of cases. We believe that bone fracture at the site of endoprosthesis stem implantation and aseptic loosening of stem takes place due to the effect of radiotherapy on sound bone, which is located in close proximity to the tumor. We evaluate the post-radiation ulcer of the skin in Patient G, as the effect of inadequate dose of radiotherapy on the skin. Several methods of eliminating complications of periprosthetic infection were as follows: removal of the endoprosthesis, installation of a metal-cement spacer followed by repeated joint endoprosthesis replacement; in cases of bone fractures at the site of endoprosthesis stem implantation it included metallic osteosynthesis with periosteal plates and cable grip; repeated joint endoprosthesis replacement was performed in cases of aseptic loosening of stem; revision of the wound, removal of necrotic tissue and closure of the wound by moving the Gastrocnemius medial head with subsequent free skin grafting was carried out in case of postradiation ulcer, hip amputation or disarticulation was carried out in case of recurrence;

*Fig. 4. Patient G. Area of the knee joint with post-radiation ulcer*

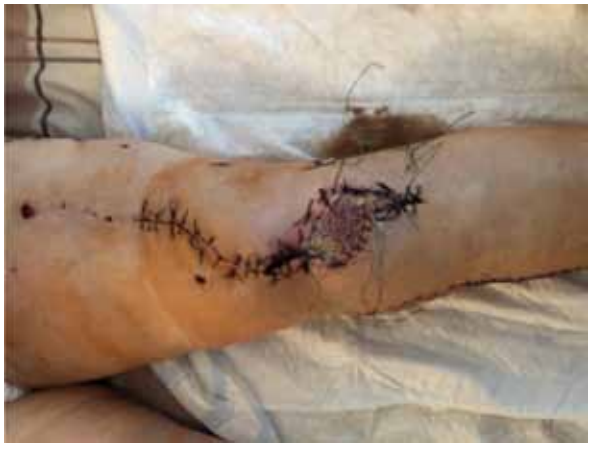

*Fig. 7. Patient G., Free skin grafting of the skin defect on the anterior surface of the lower leg with autodermal graft*

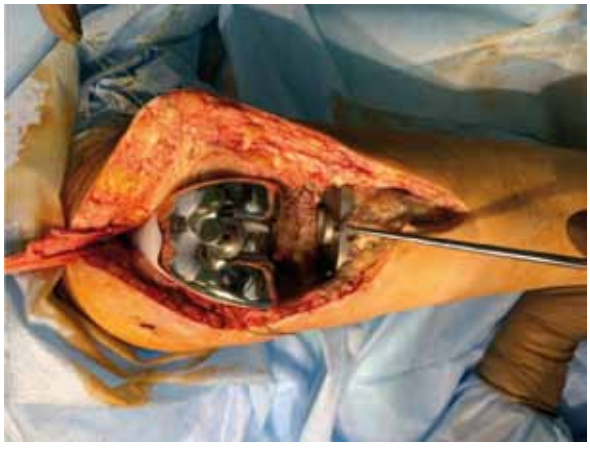

*Fig. 5. Patient G., revision of the knee, removal of necrotic tissue.*

*Fig. 6. Patient G., transposition of the medial gastrocnemius to the anterior surface of the lower leg to cover the soft tissue defect*

multiagent chemotherapy with metastasectomy was performed in case of metastases in lung. According to the literature, post-radiation damage to bones is usually accompanied by postradiation changes in skin and subcutaneous tissue [3, 11]. The main task of surgical repair regarding patients with local post-radiation lesions includes a radical excision of the diseased tissues and adequate replacement of the defect. In such cases, it is advisable to use myocutaneous flaps and muscle flaps [10]. Patients having malignant tumor of bone, who underwent the complete multimodal treatment phase, were recommended to undergo case follow-up every 3 months for the first 2 years, then every 6 months for the next 3 years. According to some researchers, mandatory examinations shall include computed tomography of thoracic cavity, x-ray examination of the implant intallation area, and ultrasonic investigation of the postoperative cicatrix [7]. Case follow-up is done to detect early orthopedic complications, instability of endoprosthesis stem, recurrence and metastases of the tumor and to address the further treatment strategy: to conduct chemoradiation or surgical treatment of recurrent tumors and resectable metastatic sites.

## CONCLUSION

1. The use of multiagent chemotherapy arises complications during knee replacement, such as periprosthetic infection in 7.7% of cases due to a decrease in the patient`s phylactic power. The main strategy for eliminating complications of periprosthetic infection included removal of the endoprosthesis, installation of a metal-cement spacer, followed by repeated joint endoprosthesis replacement.

2. The use of radiotherapy also arises complications, such as periprosthetic infection, bone fracture

at the site of endoprosthesis stem, aseptic loosening of stem and post-radiation ulcer due to the impact of radiotherapy on the skin and sound bone in the immediate vicinity of the tumor.

3. Surgical treatment of postradiation osteomyelitis, fibrosis and ulcers involves extensive excision of all affected tissues in a single block, and replacement of soft tissue defects with myocutaneous flaps and muscle flaps.

### *Conflict of interest*

This paper does not cause any conflict between the authors, has not been and will not be the subject of commercial interest or reward in any form.

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# ANALYSIS OF POSTOPERATIVE COMPLICATIONS IN REPAIR OF INCISIONAL VENTRAL HERNIAS USING ALLO-AND AUTOGRAFTS

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**ABSTRACT** — The article provides a multicenter retrospective analysis of postoperative complications in patients after ventral hernias repair. The study went on for 10 years and included 628 patients divided into 2 groups. The first group included 510 patients operated on using polypropylene implants (mesh density 60g/m$^2$). In the second group (118 patients) a de-epithelized autodermal graft prepared according to the authors' technology was applied. Clavien-Dindo classification was used to describe postoperative complications. The total number of complications in the first group was 22.2%, in the second group — 2.5% ($p < 0.01$). In the analysis of complications in all patients operated on using both allo- and autograft, the most frequent complications in the form of seroma were noted when implants using the one lay technique — 22.3% ($p < 0.05$) were installed. The autodermal de-epithelized graft used in ventral hernia plastics, regardless of their localization and size, especially in relapses after alloplasty, may be a good alternative to synthetic grafts.

**KEYWORDS** — incisional hernia, postoperative complications, abdominal wall repair, mesh implants, autodermal graft, recurrent hernia.

## INTRODUCTION

Intervention for incisional hernia is the most commonly performed abdominal surgery, which requires extremely high economic costs. The risk of complications after hernioplasty is a key parameter in estimating the risk/benefit ratio for evaluating the technique. According to hospitalization studies, one in five patients who underwent incisional hernia plastic in the USA was re-hospitalized within a year, with the majority of repeated hospitalizations occurring in the 30-day post-surgery period [1]. The analysis, published in the Journal of Surgical Research, indicates that many patients who are re-hospitalized with complications after surgical hernia plastics are often ignored, leading to a constant underreporting of complications [2, 3, 4]. Hernia plastic includes two types of materials: synthetic and biological implants. Synthetic mesh remain in the body for life, provide adequate mechanical support, but are associated with postoperative complications such as infection. Biological implants are obtained from xenografts or their own tissues less susceptible to infections; however, their mechanical strength may be too weak depending on the characteristics of the hernial defect [5, 6, 7]. Unfortunately, recurrent hernia is itself a risk factor for repetition of subsequent surgeries, meaning that many patients are in the vicious circle of numerous failed surgeries. The risk of complete mesh excision may be significant, often requiring extensive lysis of adhesions with a risk of intestinal damage. In addition, if the mesh is well embedded, there is a risk of destruction of the natural components of the abdominal wall, which makes subsequent reconstruction difficult [8, 9]

### *Purpose*

The purpose of the study was to analyze early and late postoperative complications when using allo- and autografts in the surgery of incisional ventral hernias.

## MATERIALS AND METHODS

A multicenter retrospective study of the treatment outcomes in 628 patients was conducted. The first group — 510 patients were operated with polypropylene implants (polypropylene, mesh density 60 g/m$^2$) and the second group — 118 patients using a de-epithelized autodermal graft prepared according to the author's method. To prepare the graft, the skin was taken from the surgical access area of the same patient, mechanically de-epithelized and impregnated with an antibiotic solution and an oxygen-containing preparation. The groups were comparable in sex, age and pathology. The arrangement of implants in the first group was as follows: one lay — 310, sub lay — 190 and in lay — 10, in the second group: one lay — 61, sub lay — 55, and in lay — 2. According to the EHS classification in the first group, the distribution was as follows: M2W3R1 — 133, M3,4W3R1 — 121, M4,5W3R1 — 89, L1W3R2 — 66, L3W2R1 — 54,

L4W3R1 — 47. In the second group: M2W3R1 — 41, M3,4W3R1 — 37, M4,5W3R1 — 18, L1W3R2 — 11, L3W2R1 — 7, L4W3R1 — 4. All patients with recurrent hernias after alloplasty were operated with autografts. The study lasted 10 years. Clavien-Dindo classification was used to describe postoperative complications. Statistical processing of the digital material of the dissertation was carried out using the STATISTICA Version 6 program. In the process of statistical analysis, quantitative values are indicated as the average statistical value (M) ± standard quadratic deviation (SD). Statistical processing of digital data when determining the validity of differences in quantitative values of results is performed using Student's t-test.

## RESULTS AND DISCUSSION

Early complications in the first group were distributed as follows: seromas — 26, hematomas — 11, suppuration — 6, paresis — 5 (9.44%). In the second group, early complications were represented by only 2 seromas (1.7%). Late complications in the first group were distributed as follows — fistula formation — 15, infiltrates and abscesses — 7, graft detachment and migration — 10, neuralgia and paresthesia — 18, recurrence — 15 (12.75%). Late complications in the second group were presented by 1 case as a relapse 2.5 years after surgery (0.8%). The total number of complications in the first group was 22.2%, in the second group — 2.5%. When analyzing complications using polypropylene grafts according to the one lay method — 101 (19.8%), sub lay — 13 (2.5%), in lay — no complications were noted. All complications of the second group arose in the patients operated on by the one lay technique. In the analysis of complications in all patients operated with both allo- and autografts, the most frequent complications in the form of seroma were noted when implants were installed using the one lay technique — 22.3%. When implants were installed using the sub lay technique, complications in the form of hematomas and suppuration were noted in single cases (0.8%), and the most frequent complication in this case was GI paresis — 10.2%. Suppurative processes in the form of fistulas, infiltrates and chronic abscesses in the first group were noted in 5.4% of cases. General data are presented in the Table 1.

Analysis of the results of treatment of ventral postoperative and recurrent hernias using allo- and autografts in the study groups showed that sub lay is the optimal method of their fixation. The largest number of complications was noted in the one lay technique using a polypropylene implant. The obtained data correlate with the results of foreign studies [10]. During the analysis, it turned out that the use of an autograft, regardless of the location of the implant, gives a minimum number of complications both in the early and late postoperative period.

***Table 1.*** *Analysis of postoperative complications in comparison groups*

| Degree of complications by Clavien-Dindo | Study Groups | | P |
|---|---|---|---|
| | I group (n = 510) abs (%); (M ± SD) | II group (n = 118) abs (%); (M±SD) | |
| I | 29 (5,6%) | 2 (1,7%) | < 0,05 |
| II | 18 (3,5%) | abs | - |
| IIIa<br>IIIb | 16 (3,1%)<br>27 (5,3%) | 1 (0,85%)<br>abs | < 0,05 |
| IVa<br>IVb | 17 (3,3%)<br>7 (1,4%) | abs<br>abs | -<br>- |
| V | abs | abs | - |
| Total | 114 (22,2%) | 3 (2,5%) | < 0,01 |

## CONCLUSION

The autodermal de-epithelized graft used in ventral hernia plastics, regardless of their localization and size, especially in relapses after alloplasty, may be a good alternative to synthetic grafts.

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# ADHESIOGENESIS OF RESIDUAL LIVER CAVITIES AFTER ECHINOCOCCECTOMY

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**ABSTRACT** — This paper presents findings of the retrospective analysis of 58 cases of surgical treatment in patients with hepatic hydatidosis. In all patients after open or laparoscopic echinococcectomy drainage of the residual cavities was performed. We developed and applied our own technique of obliteration of the residual cavities in all patients. Complete obliteration of the residual cavity occurred in 10–15 days after the surgery. The postoperative bed-day was 12.5. All patients recovered and were discharged in satisfactory condition.

**KEYWORDS** — echinococcosis, liver, surgery, povidone-iodine, residual cavity.

## INTRODUCTION

The hydatid form of echinococcosis is an urgent problem due to the consistently high incidence rates among the population of our country. The liver is the organ with the most frequent localization of the pathological process [1, 2, 3, 4, 5]. Currently, the most popular method of treating echinococcosis is a surgical intervention. One of the most common surgical options for treatment of echinococcosis is open echinococcectomy [6]. This operation, from the very beginning to its present numerous modifications, remains a minimally traumatic procedure. It characterizes by minimal blood loss both during the operation and after it. Echinococcectomy can also be performed laparoscopically: using modern diagnostic techniques, enabling in most cases to avoid blood transfusion and blood substitutes. Due to the fact that the cavity of the cyst can be large and gigantic in size, the healing progresses slowly with the risk of fistula formation, laparoscopic echinococcectomy is undertaken with caution.

To date, various methods of eliminating residual cavities have been proposed: from conservative to surgical. The most popular are the methods of conservative treatment of residual cavities. However, despite this diversity, none of the existing methods meet the expectations of surgeons.

*Purpose of the study:* improving the results of surgical treatment of patients with hepatic hydatidosis

## MATERIALS AND METHODS

We analyzed the case histories of 58 patients who underwent either open or laparoscopic echinococcectomy in the Department of Surgical Diseases at Astrakhan Medical University (Russia). A technique developed by the authors for obliteration of residual cavities was used in all patients. The technique consisted of daily washing the residual cavities with a 10% povidone-iodine solution in the morning and in the evening during the bandaging of patients. Then the drainage was pinched — an exposure was created for 15 min. At the same time, in 53 (91.4%) patients the obliteration technique was applied after open echinococcectomy and in 5 patients (8.6%) — after external drainage of the residual cavity, indications for which were: intrahepatic location of single irregular echinococcal cysts; the impossibility of eliminating the residual cavity by suturing tightly, due to technical difficulties and the risk of damaging hepatic anatomical structures.

There were 1.5 times more male than female patients. Susceptibility to echinococcosis was observed in the following age groups: at the age from 41 to 50 years — 36 patients (62.1%), from 51 to 60 years — 18 patients (31%). Localization of echinococcal cysts was predominantly in the right lobe of the liver — 39 (67.2%), while in the left lobe — 19 (32.8%). The size was of echinococcal cysts ranged from 10 cm in diameter — 26 (44.8%), 20 cm — 25 (43.1%), and a few cases of 30 cm in diameter — 7 (12.1%).

## RESULTS AND DISCUSSION

Open echinococcectomy was completed with drainage of the residual cavities. Two polyvinyl chloride tubes 24–27 Fr with lateral holes were installed in the residual cavities after echinococcectomy, and two drainage tubes were inserted into the subhepatic and subphrenic spaces to control hemobilia. Starting on the same day after the surgery, the technique proposed by the authors was applied. Then the drainage was pinched — an exposure was created for 15 min. No allergic reactions to the drug administration were reported. There were no cases of bleeding in the postoperative period. 9 (15.5%) patients had complications.

The procedure for introducing a 10% povidone-iodine solution was performed until the cyst cavity was completely obliterated. Obliteration of the residual cavity occurred 10–15 days after the operation. The postoperative bed-day was 12.5. All patients recovered and were discharged in a satisfactory condition. Monitoring clinical and biochemical blood parameters showed no significant changes in the red blood cells. This confirms that such operations were well tolerated by the patients and demonstrated a minimal risk of intra- and postoperative complications. Moderate leukocytosis after surgery and its decrease by day 7 indicates the anti-inflammatory and antiseptic effect of povidone iodine. Among the studied biochemical parameters, ALT, AST fluctuations attracted attention. Their values reached their maximum level on day 1 after surgery: ALT — 1.55 ± 0.2 ($p<0.05$) mmol/l, AST — 0.95 ± 0.3 ($p<0.05$) mmol/l. Their normalization occurred on the 7–8$^{th}$ day after the operation. The rest of the indicators remained within the normal range. The increase in ALT and AST indicators, in these cases, is not associated with the toxic effect of drugs, but is due to the liver surgery itself. This explains the reactive increase in transaminases. A low invasiveness of the operation facilitates the normalization of the level of transaminases.

In order to study the dynamics of obliteration of the residual cavities, fistulography, ultrasound and computed tomography of the residual cyst cavities were carried out. Fistulography was performed 7–14 days after the surgery. X-ray diffraction patterns showed a progressive decrease in the residual cavities, and by 14 days the cavity is no longer contrasted. The results of the ultrasound study of the residual cavities in the postoperative period coincide with the data of fistulography and CT studies of the residual cavities.

## CONCLUSIONS

1. The applied drug povidone-iodine 10% is an antiseptic with a wide spectrum of action.
2. Good adhesive properties of the proposed method of obliteration help to achieve effective and rapid healing of the residual cavity, which in its turn reduces the postoperative hospital stay and the period of temporary disability.
3. The developed method of obliterating residual liver cavities after echinococcectomy proved to be simple, accessible and effective and can be recommended for clinical practice.

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# THE SIGNIFICANCE OF PHARMACOGENETIC TESTING FOR BETTER ANAESTHETIC OUTCOME AND LESS SURGICAL STRESS. LITERATURE REVIEW

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**ABSTRACT** — The review is devoted to the problem of optimizing the anesthetic manual based on pharmacogenetic data in order to achieve an adequate depth of anesthesia and stress protection and reduce the number of adverse drug reactions. We analyzed the data of Pub Med and Web of Science databases to investigate the influence of genetic polymorphism on the body's response to the main groups of drugs used for anesthesia, and changes in the effects of drug interaction. Specifically, we have reported that the use of preoperative genetic screening for a set of markers (polymorphic alleles of a number of cytochromes) is a promising tool in the anesthesiologist's practice.

**KEYWORDS** — pharmacogenetics, anaesthetic manual, surgical stress, genetic polymorphism.

## INTRODUCTION

The main objective of the anaesthetic manual is to achieve an optimal level of sedation, analgesia and neurovegetative protection in order to reduce surgical stress and associated perioperative complications. Surgical stress response is a complex of neurometabolic, neuroendocrine, and inflammatory disorders that develops as a result of surgical trauma [5]. If it is not prevented by its own stress-limiting systems (GABA, opioid peptides, antioxidant system, and others), it leads to perioperative dysfunction of organs and systems [5, 10]. This condition is regarded as *insufficient anesthesia*. However, complete blockage of all reactions to trauma, the so-called *stress-free anesthesia*, is accompanied by a lot of complications, significant respiratory depression, and requires prolonged artificial ventilation. That is, the question of in what casesthe extent to which suppression of the response to chemical stress is desirable remains unresolved [12]. The modern anaesthetic manual is based on the principle of multimodality (multi-component), since no component of anesthesia is able to provide the necessary level of anti-stress protection. The variability of the patient's response to the components of anesthesia and, accordingly, its adequacy in 20–95% of cases is determined by the genetic polymorphism of the substrates of their pharmacokinetics and / or pharmacodynamics (metabolic enzymes, receptors, transport systems, etc.), which naturally affects the effects of drug interaction. Projected the specialist anesthesia for the patient in this case, it often may not provide a sufficient level of depth of anesthesia or accompanied by manifestations of undesirable, toxic drug reactions, or to increase the frequency of their manifestation and severity. From these positions, it is necessary to search for new ways to optimize the anesthetic manual, personalize the selection of components and dosages of drugs used [9, 3]. The use of a personalized approach using *omix* technologies and advances in pharmacogenetics in the future will allow using the most effective drug in a sufficient dose and safe from the point of view of undesirable drug reactions (NLR) [13].

## METHODS

The analysis of published data, including in the Pub Med and Web of Science databases, on the influence of genetic polymorphism on the body's response to the main groups of drugs used for anesthesia, and changes in the effects of drug interaction. 118 sources were analyzed from 2005 to 2018, and 22 sources are included in the article.

## PHARMACOGENETICS: RELEVANCE FOR THE ANAESTHETIST

Neuroendocrine changes are the basis of complex reactions of the body to surgical trauma. From the perspective of a comprehensive and preventive effect on surgical stress, it is relevant to assess the genetically determined changes in the pharmacodynamics of the most significant groups of drugs in the anesthesiological support for the development and limitation of this process. This is especially important due to the peculiarities of anesthesiological practice and the need to use a wide range of highly active drugs from different

pharmacological groups in high doses as part of a single manual. Taking into account the pharmacogenetic features of the kinetics and mechanism of action of drugs and their altered interaction with each other will help to ensure the sufficiency and safety of anesthesia components [1, 15]

In accordance with the pathogenesis of surgical stress, the most significant components of an anesthetic aid are sleep, analgesia, and correction of autonomic disorders, which are provided by the use of such groups of drugs as General anesthetics, hypnotics, opioid analgesics, neuroleptics, and others.

Currently, volatile liquids (fluorotane, enflurane, sevofluan, influrane) and gaseous substances (nitrous oxide, xenon) are used from the group of inhaled General anesthetics. The pharmacokinetics of gaseous agents practically excludes the effect on their metabolism (xenon does not undergo biotransformation, nitrous oxide is only 0.01% metabolized in the gastrointestinal tract). In this case, xenon has an effect on the inflammatory component of the surgical stress response.

According to some data, from 20 to 45% of fluorotane undergoes hepatic metabolism with the participation of cytochrome P450 CYP2E1, being oxidized to trifluoroacetic acid (TRIFLUOROACETYL chloride binds to liver proteins and triggers an autoimmune response), chlorine and bromine ions, and under hypoxia is restored with the formation of hepatotoxic products. However, there are no data on common mutations of the CYP2E1 gene that are accompanied by changes in metabolism and contribute to the development of halothane-induced hepatitis. the activity of the enzyme depends more on the properties of the phenotype and dietary characteristics [4]. Repeated contact with halothane increases the risk of necrosis and increases the associated mortality. An inhibitor of the CYP2E1 isoenzyme is disulfiram, which reduces the formation of the metabolite and may reduce the risk of halothane hepatitis. There are experimental data on the ability of benzodiazepines, a number of which are used as premedication components (diazepam, nitrazepam, midazolam), to also have an inhibitory effect on the activity of the CYP2E1 enzyme in micromolar concentrations. Their effect on the development of adverse drug reactions (NLR) of halothane in the form of hepatitis has not been studied.

In addition, halothane itself can act as an inducer of the metabolism of phenobarbital and other barbiturates and thus reduce the effectiveness of sodium thiopental used as a hypnotic.

Hepatic metabolism and the risk of hepatitis in other volatile anesthetics is significantly lower (isoflurane 0.17%, desflurane 0.01%, sevoflurane 1–5%, enflurane 2.4% is metabolized in the liver). Sevoflurane does not produce acylated protein compounds; the main product of sevoflurane degradation under the action of bases is nephrotoxic vinyl ether (compound A) [11].

The leading role in the metabolism of the intravenous anesthetic ketamine (calypsol), its conversion to norketamine is played by the enzymes CYP2B6, CYP3A4, and CYP2C9 (the latter two are auxiliary). The genetic polymorphism of these enzymes has no convincing evidence of influence on the drug's action. But the parallel use of ketamine with inducers or inhibitors of cytochromes significantly affects its metabolism. Inhibitors of CYP3A4, CYP3A6, SUR2C19, SUR2B6, SUR2C9, antibodies to these cytochromes and their common inhibitor aminobezotriazole block the metabolism of calypsol and reduce the rate of its D-methylation. In connection with the above, the ability of pharmacological components of anesthesia to influence the activity of processing processes of this intravenous anesthetic has prospects for study [8].

Hypnotics are drugs that promote sleep, but their effect is not accompanied by analgesia. These are barbiturates (sodium thiopental, hexenal), benzodiazepines (diazepam, midazolam) and propofol.

Barbiturates are currently used as part of drugs for the induction of anesthesia. Compounds of pharmacologically inert barbituric acid with oxygen make up the group of oxybarbiturates (hexobarbital), compounds with sulfur — thiobarbiturates (sodium thiopental). The metabolism of the former occurs only in the endoplasmic reticulum of hepatocytes, the latter also have extrahepatic metabolism (CNS, kidneys) [12]. Transformation of barbiturates occurs in several stages: hydroxylation in the liver with the participation of NADH and P450 with the formation of oxyceto- and carboxybarbituric compounds, dealkylation, destruction (hydrolysis opening) of the barbituric ring, in thiopental — desulfurization with the formation of phenobarbital, pentobarbital. The P450 isoforms CYP2C19 and CYP2C9 have the highest value in the transformation. Not only barbiturates are metabolized by CYP2C19 (S-mephenytoin hydroxylase), but also many other widely used drugs (benzodiazepines, beta-blockers,proton pump inhibitors, and others), about 8% of all registered drugs. Today, 17 variants of CYP2C19 alleles are known, which are more or less associated with a decrease in protein expression, a decrease or lack of functional activity of the enzyme, and a different distribution of polymorphic variants in different ethnic groups [6, 7]. This multisubstrate metabolism contributes to the inter-drug interaction. Barbiturates have the property of self-induction against CYP2C19. The significance of the polymorphism of this gene in the practice of using barbiturates

as part of an anesthetic aid and the effect on drug interaction requires further study. The situation is similar for CYP2C9 substrates [12].

Benzodiazepines are mainly used for premedication, anxiolysis, amnesia, and sedation. Currently, in most countries of Europe and the United States, three benzodiazepine receptor stimulators of this structure are used in anesthesiology — midazolam, diazepam and lorazepam. Most benzodiazepine derivatives are metabolized by liver enzymes of the cytochrome P-450 system to polar compounds and are excreted in the urine and bile [4]. The metabolism of benzodiazepines is an example of how each substrate can be processed by several enzymes. According to the results of numerous studies, the isoenzymes CYP3A4, CYP3A5, and CYP2C19 are involved in the biotransformation of these substances. There are also indications of a possible role for CYP2D6 and CYP2C9. Polymorphisms of the CYP2C19 and CYP2C9 genes are significantly associated with the risk of adverse events (NLR) when using benzodiazepines as tranquilizers [8].

Diazepam is converted to temazepam by hydroxylation with the participation of CYP3A4 (nonlinear dependence), and then to N-dimethyldiazepam by dealkylation under the action of CYP2C19 (according to the Mikhail-Menkes system). The ratio of metabolites is 4/1. The kinetics of diazepam is influenced by the patient's gender, age and weight, and functional safety of the liver, but a significant effect of the CYP2C19 gene polymorphism has also been reliably established. The half-life of diazepam in *fast metabolizers*, homozygotes for the A-allele G681A polymorphism of this cytochrome is 4 times higher than in most *slow metabolizers*. In individuals who are heterozygous for the A-allele, the half-life varies between these two values [4, 8]. *Slow* genetic variants or the use of CYP2C19 inhibitor drugs (proton pump inhibitors, paroxetine, and others) may be clinically manifested by prolongation or deepening of sedation after diazepam administration. Since diazepam has a dose-dependent respiratory depression, there may be an increase in cases of apnea on the introduction of an induction dose [8].

At the same time, genetically determined accelerated metabolism or the use of inducer drugs (carbamazepine, barbiturates) can theoretically be accompanied by insufficient effectiveness of diazepam as a hypnotic for induction anesthesia. Benzodiazepines have a greater value as the stress-limiting preparations, compared to inhaled anesthetics and barbiturates. Their use significantly limits the increase in cortisol concentration, since their mechanism of action affects not only the hypothalamic-pituitary system, but also direct suppression of the synthesis of glucocorticoids is not excluded. Accelerated metabolism may affect the amnesic effect of the drug and contribute to the preservation of unpleasant memories in the postoperative period [8].

Unlike diazepam, the clinical response to midazolam is poorly associated with genetic factors. CYP3A4 and CYP3A5 polymorphisms are associated with a decrease in midazolam clearance, but these associations are not sufficiently pronounced to indicate a clinical difference due to the existence of alternative pathways of metabolism and excretion [8].

Based on clinical observations of the reactions of patients of the same ethnic origin, significant individual susceptibility to the intravenous anesthetic propofol was determined, and this variability is reflected in the different dose requirements and time required for recovery. Based only on the traditional algorithm for calculating the dose of propofol, it is very difficult for patients to get adequate anesthesia. Deep sedation with propofol, which suppresses the stress response, is accompanied by hypotension. Sedation-related complications or even brain trauma can subsequently worsen the outcome of surgery in patients. Similarly, insufficient propofol sedation, defined as inadequate anesthesia, would cause hypertension, tachycardia, or movement of the patient and lead to intraoperative consciousness. The etiology of individual variability of the response to propofol may be influenced by genetic polymorphisms of its metabolic enzymes or structures involved in the implementation of the pharmacological effect. The effect of the CYP2B6 polymorphism on propofol pharmacodynamics and General anesthesia has not been sufficiently studied [8].

Studies evaluating propofol sensitivity have only targeted the CYP450 genes that are involved in propofol metabolism. Murana and co-authors found that a polymorphism in the CYP2B6 gene (rs3745274) affects sensitivity to propofol anesthesia. Studies by Lian and others have demonstrated that the influence of CYP2C9 polymorphism also contributed to changes in susceptibility to propofol. These observations6 have shown that the enzymes involved in the metabolism of this hypnotic affect the susceptibility to it and that the total consumption of propofol can be disrupted by other substances, inducers or inhibitors of these enzymes used during surgery [23].

The pharmacodynamics of propofol also changes under the influence of receptor polymorphism. Researchers from China, using the Sequenom MassARRAY single-nucleotide polymorphism (SNP) genotyping method, identified a mutation (rs6313) in the 5HT2A receptor gene that correlated with individual propofol sensitivity, concentration, and induction start time. Carriers of the minor allele (G) 5ht2a rs6313

required less propofol (20% reduction in concentration) and less time (40% reduction in start time) to induce anesthesia, and they show stronger activation of sleep-stimulating neurons in the ventrolateral preoptic region (VLPO), which contribute to anesthetic hypnosis. This result may significantly contribute to elucidating the role of 5HT2A receptors in sensitivity to propofol anesthesia.

Binding to agonistic GABA sites is thought to contribute to the hypnotic effect of propofol. GABA receptors and M2 cholinergic receptors contribute to cardiovascular susceptibility to propofol anesthesia. A g-to-A mutation in the rs2279020 gene in the GABA receptor can alter its pharmacological properties by changing the composition and location of subunits. When anesthetized with propofol, the minor a allele can cause a stronger inhibition in the brain in the carrier, which is manifested by higher BIS rates in these patients after loss of consciousness [23].

Dominant mutations in the genes GOMKA1 rs2279020, GABKA2 rs11503014, and the M2 holinoreceptor gene rs1824024 may presumably be associated with a predisposition to cardiovascular complications during propofol anesthesia. Propofol significantly reduced heart rate against the background of loss of consciousness in patients who were carriers of the minor g allele rs11503014 in GABA-2, compared with patients without the g allele. Variation of the C-To-a rs2283265 polymorphism in HRM2 resulted in lower heart rate values. Changes from G to a rs2279020 in GABAA1 are accompanied by a lower BP curve after propofol anesthesia [23].

Clinically significant concentrations of propofol alter the functions of potential-dependent sodium channels, thereby inhibiting the release of glutamate from presynaptic endings. The SCN9A gene, which encodes the Nav1.7 sodium channel, is associated with various pathophysiological conditions, such as human sensitivity to pain [34]. A Chinese study confirmed the significant role of SCN9A in propofol sensitivity. Patients who were heterozygous or homozygous for the minor allele (a) rs6746030 in SCN9A recorded significantly lower BIS values . Changing rs6746030 in SCN9A from G to A can change the function of the sodium channel; changes in glutamate release and intrinsic ionic conductivity resulted in greater susceptibility to propofol, which is shown by significantly lower BIS values after propofol-induced unconsciousness. This result may help clarify the role of SCN9A in propofol sensitivity [23].

Thus, if only the traditional propofol dose calculation algorithm is used, patients with the minor allele (G) rs6313 in 5HT2A, homozygous carriers of the main allele (GG) rs2279020 in GABA1, and carriers of the minor allele (A) rs6746030 in SCN9A are highly likely to suffer from drug overdose. This result is a prerequisite for the introduction of preoperative genetic screening to identify individuals with a high risk of excessive sedation and vascular complications during propofol anesthesia [23].

Numerous data indicate the ability of opioid analgesics to limit the severity of endocrine-metabolic changes in the stress response. Endogenous opioids are an essential part of the antinociceptive system, but the genes of opioid receptors are characterized by polymorphism [20].

Strong opioids, complete opioid receptor agonists, are represented by morphine, hydromorphone, oxycodone, Oxymorphone, fentanyl, and methadone. Fentanyl-a synthetic opioid often used during surgical procedures, causes a rapid analgesic effect when administered intravenously, easily penetrates the blood-brain barrier and demonstrates 200 times greater effectiveness than morphine. Intravenously administered fentanyl is also relatively short-acting, as it is rapidly metabolized by CYP3A4 to norfentanyl [2]

Genetic differences in patients can significantly change the pharmacokinetics of painkillers, an example of which is the mixed-action analgesic tramadol. It, like codeine and hydrocodone, is essentially a *prodrug* that requires transformation to produce a more active metabolite. In the first phase of tramadol biotransformation reactions, demethylation occurs with the participation of the P-450 CYP2D6 isoenzyme [2]. As a result, most of the drug forms O-desmethyltramadol, which has a much higher analgesic activity, the remainder is converted to inactive N-desmethyltramadol via CYP2B6 and CYP3A4. Polymorphisms of the CYP2D6 gene can significantly change the rate of biotransformation and affect the effectiveness of tramadol use, which has been convincingly demonstrated in studies of postoperative pain management in gynecology [16, 21]. Four CYP2D6 phenotypes were identified: slow metabolizers (activity score 0), intermediate metabolizers (activity score 0.5), extensive metabolizers (activity score 1.0–2.0), and ultra-lipid metabolizers (activity score >2.0). Carriers with two null alleles in the CYP2D6 gene or a combination of one null allele with a second allele with reduced function are characterized by reduced or completely absent enzymatic activity of the 2D6 isoenzyme (5–10% of Europeans). These patients are characterized by rapid accumulation of narcotic analgesic, and they need to prescribe lower doses [17].

The frequency of polymorphisms C100T and G1846A of the CYP2D6 gene of the cytochrome P-450 isoenzyme, which significantly reduce the effectiveness of analgesia and contribute to severe

sympathicotonia, can reach 30 % in different races and populations [2, 30, 29, 26]. Ultrafast metabolizers of CYP2D6 substrates are potentially susceptible to life-threatening levels of the active metabolite O-desmethyltramadol, which cause respiratory depression and neurotoxicity [2, 21].

The CYP3A4 enzyme is responsible for N-demethylation of opioids such as tramadol, fentanyl, and oxycodone to the inactive metabolites N-desmethyltramadol, norfentanyl, and noroxicodone, respectively. CYP3A4 is relatively non-polymorphic with 41 allelic variants described, of which nine have insignificant enzymatic activity in in vitro studies. Many of these variants are extremely rare, making it difficult to evaluate them in a clinical context. However, several in vitro and in vivo studies have shown that the CYP3A4*1G variant is associated with a lower fentanyl metabolic rate and significantly lower consumption compared to patients with wild-type alleles, which should be taken into account when selecting a dose for adequate pain management [16].

The metabolism of narcotic analgesics with the participation of cytochrome P450 determines the risk of drug interactions with substrates that are inducers or inhibitors of this liver enzyme system, which must be taken into account during anesthesia. All drugs that are metabolized with the participation of the 2D6 isoenzyme are potential substrate inhibitors. The inhibitory effect of substances such as grapefruit juice bergamot, ondansetron, captopril, carvedilol, tamoxifen, tamsulosin, metoprolol, nifedipine, amitriptyll and others, as well as the presence of true CYP2D6 inhibitors that are not substrates and are not metabolized with the participation of the 2D6 isoenzyme [22], such as amiodarone, celecoxib, metoclopramide, paroxetine, sertraline, ticlopidine, venlafaxine, fluoxetine and others [2]

The opioid response is mediated by corresponding receptors (μ, κ, and δ) in the Central nervous system, which interact with endogenous and exogenous opioids via G-proteins, resulting in reduced transmission of nerve impulses and inhibition of neurotransmitter release. The OPRM1 gene encoding the mu-opioid receptor may contain more than one hundred single-nucleotide polymorphisms that change the structure of the extracellular part of the receptor. A number of patients carrying the OPRM1 118AvG variant, with wild-type aspartate replacement, reduced mRNA and protein expression, and reduced signal transmission efficiency, have a reduced response to the narcotic analgesics fentanyl and Alfentanil. According to research results, they require higher doses of drugs for pain relief in the early postoperative period and, accordingly, they can have the same effect intraoperatively [16].

Some authors describe an increase in cortisol and ACTH levels in the first hour and a half after naloxone administration in individuals with genotypes 118G/G and 118A/G, as well as they were more likely to experience early postoperative complications in the form of nausea and vomiting.

## DISCUSSION AND CONCLUSION

The results of current research linking polymorphisms to differences in opioid response are promising. It is hoped that future research involving a large number of SNPs in genes that determine both pharmacokinetic and pharmacodynamic parameters will one day lead to the personalization of opioid therapy in order to maximize the analgesic effect while minimizing the risk of adverse events [2].

The use of drugs in anesthesiology has significant differences from other types of pharmacotherapy, consisting primarily in the need to often simultaneously use combinations of several substances without a previous history of their appointment. The effectiveness and safety of drugs, their ability to create adequate anesthesia that reduces the severity of surgical stress and, consequently, associated perioperative complications, is determined by the features of their pharmacokinetics and pharmacodynamics, as well as various types of drug interactions. However, these factors are subject to significant variability due to genetic polymorphism of metabolic enzymes and targets in specific individuals. The above-mentioned features of anesthesiological practice do not allow applying the recommended criteria for determining the need for genetic tests in the routine practice of an anesthesiologist in the surgical Department. This problem does not have the necessary level of illumination in the published results of domestic and foreign studies. There is no conclusive evidence of the clinical utility and ability to evaluate the effectiveness of pharmacogenetic tests in perioperative practice. Further research is needed, including evaluating pharmacogenomics data and correlations with treatment outcomes. An analysis of the available literature leads to the conclusion that preoperative genetic screening for a set of markers that are significant in altered sensitivity to the most popular drugs and their combinations (such as CYP2D6, CYP3A4, CYP2B6, CYP2E1, CYP2C9, CYP2C19) may have clinical and economic effectiveness. This approach will optimize the clinical decision-making tool and individualize the dosage of drugs used to achieve an adequate depth of anesthesia and analgesia, and reduce the number of functional and biochemical tests for monitoring and timely correction of surgical stress reactions, which will have a positive impact on the duration of the recovery postoperative period.

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# STUDY OF THE INFLUENCE OF WRIST TAPPING ON ALPHA-RHYTHM SYNCHRONIZATION IN ADULTS

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**ABSTRACT** — Objective: To study the effect of hand tapping on the synchronization of the alpha rhythm in healthy adults. Materials and Methods: The study included 51 clinically healthy volunteers of working age. Results: We have shown that under the influence of wrist tapping in a state of sensory deprivation in healthy adults there is a statistically significant change in the peak frequency ($p = 0.0006$) and peak power of the alpha rhythm ($p = 0.0003$), but the width of the peak plateau remains unchanged ($p = 0.2$). This effect of wrist tapping indicates the potential for clinical use in JME, since it was previously shown that if the selected external frequencies enter into resonance with the neurons of the antiepileptic system, then an antiepileptic effect can be obtained.

**KEYWORDS** — electroencephalography, resonance, tapping, alpha rhythm.

## INTRODUCTION

Epileptogenesis and plasticity of neural networks are two interrelated phenomena, since some epileptogenic ones demonstrate a clear reorganization of neuronal connections and the growth of neuronal elements. [1]. Lüders H.O. et al. (2000) showed that the *epileptogenic focus* includes several functional zones: epileptogenic substrate, zones of irritation and onset of an attack, symptomatic, epileptogenic zone, and also a zone of functional deficit. The zone of functional deficiency is an area of the cortex, functional changes in neurons cause the appearance of neurological and neuropsychological disorders. *Breakthrough* of epileptic activity from the focus with the onset of clinical manifestations of the disease, evidence of insufficiency of antiepileptic activation mechanisms and formers of the epileptic system [2]. The mechanism of epileptogenesis is based on the function of neurons, leading to an epileptic type of information transcoding [3] and the involvement of the brain in hypersynchronous activity, as a result of which it increases its readiness to be included in auto-rhythmic activity, which is associated with the excitatory factors of neurons under the trigger.

The most important property of an epileptic focus is its determinant nature, expressed in the ability to impose its mode of operation on other parts of the brain [4]. This leads, on the one hand, to the formation of secondary and tertiary epileptic foci, on the other, to a change in the information function of neurons in the entire brain. The set of mechanisms that prevent the spread and generalization of epileptic activity is called the antiepileptic system, which is represented primarily by the structures of the brain stem, mainly its caudal part [5], as well as the hypothalamus, caudate nucleus, cerebellum, which have an inhibitory function. The activation of these structures occurs under the influence of corticofugal impulses, and they exert an inhibitory effect on epileptic activity through inhibitory collateral influences that cause hyperpolarization of cortical neurons [6]. It is also known that each epileptic seizure increases the likelihood of the next one — the pathogenesis of this phenomenon can be based precisely on the kindling effect. Understanding this mechanism directly changes the approach to antiepileptic therapy: priority must be given to starting treatment as early as possible and preventing each subsequent epileptic seizure. This phenomenon was described by A. D. Speransky [7] as *the second blow phenomenon*. On the other hand, the theory of rekindling as a defense mechanism from the standpoint of biophysical processes is consistent with the theory of resonance. Resonance (fr. Resonance, from Lat. Resono *I answer*) is a frequency-selective response of an oscillatory system to a periodic external influence, manifested in a sharp increase in the amplitude of stationary oscillations when the frequency of the external influence coincides with certain values specific to this system. With the help of resonance, even very weak periodic oscillations can be distinguished and / or amplified. Resonant phenomena can lead to both destruction and an increase in the stability of mechanical systems and, consequently, epileptic systems. Exposure to external stimuli can provoke epileptic seizures and, conversely, if the selected frequencies come into

resonance with the neurons of the antiepileptic system, an antiepileptic effect can be obtained. The theory of resonance, nowadays, is increasingly used in neurorehabilitation, when the impact of external stimuli with a certain frequency, which is in resonance with the neuronal activity of protective systems, can have a clinically significant therapeutic effect [8, 9]. Thus, using the theory of resonance and hypersynchronization, we can, by external influence at certain frequencies, desynchronize neurons (neural network) in the focus of epileptic activity, achieve the phenomenon of resonance with neurons of the antiepileptic system and reduce the risk of developing generalized and secondary generalized seizures.

In recent years, not only drug-based, but also non-drug methods of epilepsy therapy have been actively studied [10], among which the method of hand tapping is of great interest [11], which leads to a decrease in the severity of anxiety in both healthy volunteers and patients with epilepsy [12] However, we have not found any works that study the effect of wrist tapping on the severity of alpha rhythm synchronization, both in healthy people and in patients with epilepsy, which is of undoubted scientific and clinical interest.

*The purpose of this study* is to assess the effect of hand tapping according to the author's method on the synchronization of alpha activity in healthy adults.

## MATERIALS AND METHODS

The frequency spectrum of the alpha rhythm was assessed using a computer encephalographic complex ("Neurocartograf", MBN, Moscow). The following parameters of the alpha rhythm in the occipital leads (O1, O2), the minimum frequency of the alpha rhythm (Hz), the maximum frequency of the alpha rhythm (Hz), the width of the peak (Hz), the type of the peak of the alpha rhythm (monopeak, bepeak, polypeak). In addition, the power of the alpha rhythm (Hz/MkV2) in the occipital leads was analyzed. The above characteristics of the alpha rhythm were analyzed and recorded by us before the hand tapping technique and during the first three minutes after its completion. EEG recording was carried out in a state of sensory (visual and sound) deprivation. The study of wrist tapping was carried out using a modified author's technique "A method of influencing the individual rhythm of a person by means of exogenous rhythmic stimulation" (RF patent No. 2606489 dated 01/10/2017). The modification of the method consisted in the fact that the study of the individual rhythm of the subjects was carried out without the use of exogenous rhythmic stimulation. The study was carried out in the morning in conditions of exclusion of external sensory stimuli (loud sound, bright light), the presence of other people (except for a doctor and a volunteer) during the tapping technique. The temperature regime of the environment was observed in the range of 22–25° C. Tapping was performed with the subject's eyes closed. The technique consisted of striking the hand with a finger on the surface of the device (a Xiaomi smartphone based on Android, the country of origin China), followed by registration of the time parameters of this process in the author's program based on the modified technique "Method of influencing the individual rhythm of a person through exogenous rhythmic stimulation" (RF patent No. 2606489 dated 10.01.2017). A mechanogram was reflected on the screen of the device, where vertical strokes indicated the moments of contact of the finger of the hand with the smartphone screen.

***Inclusion criteria***: healthy adults; signed voluntary informed consent; male and female; age period: adolescence (m 17–21 years old; f 16–20 years old); the first period of middle age (m 22–35 years old; f 21–35 years old); the second period of middle age (m 36–60 years; f 36–55 years); Russian speaking Europeans.

***Exclusion criteria from the study***: children and adolescents; refusal to participate in this study; participation in other studies; acute and chronic neurological, psychiatric and endocrinological diseases at the time of the study; alcohol intake (2 or more drinks within the last 2 weeks); use of narcotic drugs at the time of the study and in history.

The study included 51 clinical healthy volunteers of working age (median age — 39 [21; 56] years.

Volunteers received no remuneration for participating in this study. The researchers did not receive any remuneration for conducting this study.

Statistical processing was carried out using the Statistica software package (StatSoft, version 10, USA). All data distributions were evaluated using the Shapiro-Wilk test. As a result of the study, nonparametric variables were obtained. Statistical significance was determined using the nonparametric Wilcoxon test (differences between groups were considered statistically significant at $p < 0.05$).

## RESULTS

Under the influence of wrist tapping in a state of sensory deprivation (Table 1), in healthy adults there is a statistically significant change in the peak frequency ($p < 0.001$) and peak power of the alpha rhythm ($p < 0.001$), but the width of the peak plateau remained unchanged ($p > 0, 05$). The majority of the subjects (71%) showed a change in the peak of the

alpha rhythm (Table 2), of which 43% showed its splitting and transformation into a polypeak, and 27% — a decrease in the number of peaks. Only in 29% of the surveyed the characteristics of the peak of the alpha rhythm did not change.

## DISCUSSION

According to S.N. Aksenov (2004) [13], constant movement from one unstable state to another allows living organisms to adequately adapt to constantly changing external conditions. Thus, living organisms have their own biorhythms synchronized with the external rhythms of the environment [14]. The main regulator of biorhythms and the life processes caused by them is the brain [25], the biorhythm of which is associated with the individual characteristics of self-regulation mechanisms and the level of plasticity of neurodynamic processes [16]. Considerable attention is paid to the study of synchronization of various parts of the cerebral cortex, primarily in the range of alpha and beta rhythms, in the processes of regulation and changes in the functional state of the body [17, 18]. The concept of dynamic functional connectivity is an important aspect of resting brain functional activity that looks at variations in functional connectivity over a short period of time. Dynamic functional connections are currently being explored in a variety of contexts related to both behavior and neural activity, thus deepening our understanding of functional networks in the brain. Numerous studies have shown reproducible patterns of short-term neuronal activity that travel throughout the brain [19]. Analysis of dynamic functional connections showed that spontaneous transitions between networks of interacting brain regions are highly organized into a hierarchy of two types of meta states: one for higher-order cognitive systems, and the other for sensorimotor systems [20]. New evidence is also emerging indicating that dynamic functional relationships are influenced by a variety of factors, such as mental states [21], sleep [22], learning [23], and brain disease [24]. Several studies have shown hyperdynamic activity in some specific functional networks at rest in various subtypes of epilepsy [25]. Thus, the processes of synchronization of the bioelectric activity of the brain in various physiological and pathological states of the body in active wakefulness and relaxation, as well as in the presence of anxiety, have not been sufficiently studied. Comprehensive study of the processes of synchronization of brain biorhythms both at rest and with external (exogenous) stimuli contributes to the understanding of the processes of development of plasticity of nervous processes and the regulatory function of the brain.

***Table 1.*** *Characteristics of the alpha rhythm before and after wrist tapping in healthy adults*

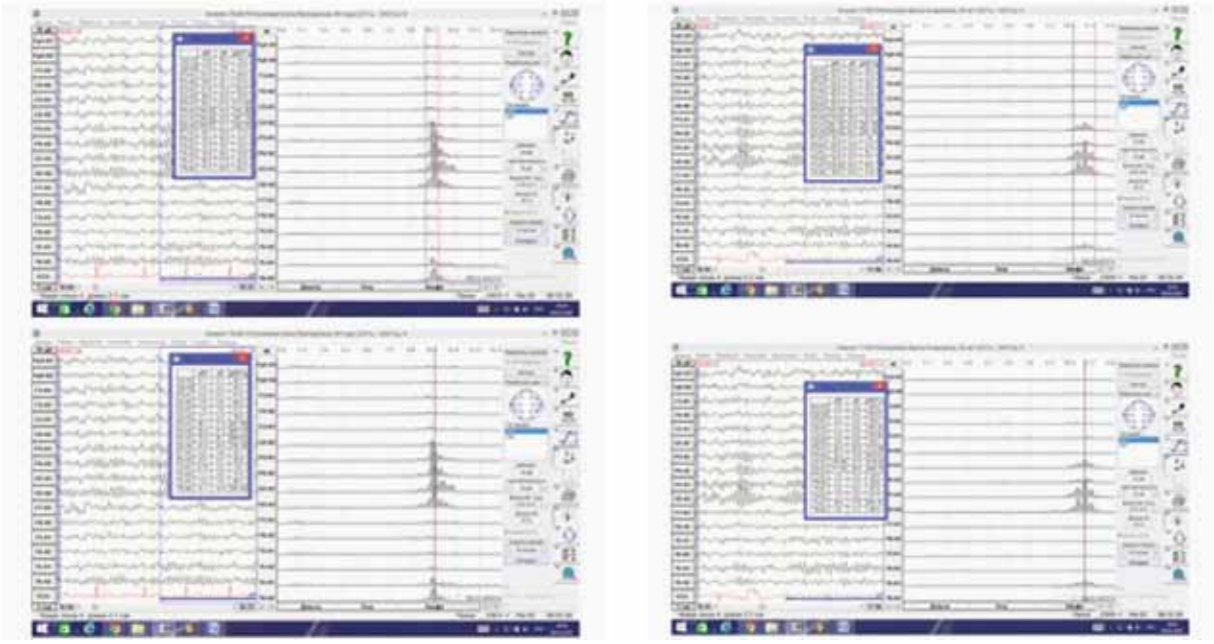
| Before tapping Me [P25;P75] | After tapping Me [P25;P75] | *p* |
|---|---|---|
| Peak frequency (Hz) | | |
| 10,2 [9,6; 10,8] | 10,6 [10; 11,1] | 0,000613 |
| Peak power (Mv / Hz) | | |
| 1357,4 [688; 2913] | 1398,172 [501; 1472] | 0,00036 |
| Plateau width (Hz) | | |
| 1,2 [1; 1,5] | 1,2 [0,7; 1,8] | 0,2 |

***Table 2.*** *Changes in the characteristics of the alpha-rhythm peak in the occipital leads before and after wrist tapping in healthy adults*

| Peak characteristic | | Amount (%) |
|---|---|---|
| Before tapping | After tapping | |
| Remained unchanged | | 15 (29%) |
| Monopeak | Monopeak | 3 (5,8%) |
| Doublepeak | Doublepeak | 5 (9,8%) |
| Polypeak | Polypeak | 7 (13,7%) |
| Increasing the number of peaks | | 22 (43%) |
| Monopeak | Doublepeak | 9 (17,6%) |
| Doublepeak | Polypeak | 6 (11,7%) |
| Monopeak | Polypeak | 7 (13,7%) |
| Reducing the number of peaks | | 14 (28%) |
| Polypeak | Monopeak | 5 (9,8%) |
| Polypeak | Doublepeak | 8 (15,6%) |
| Doublepeak | Monopeak | 1 (1,9%) |

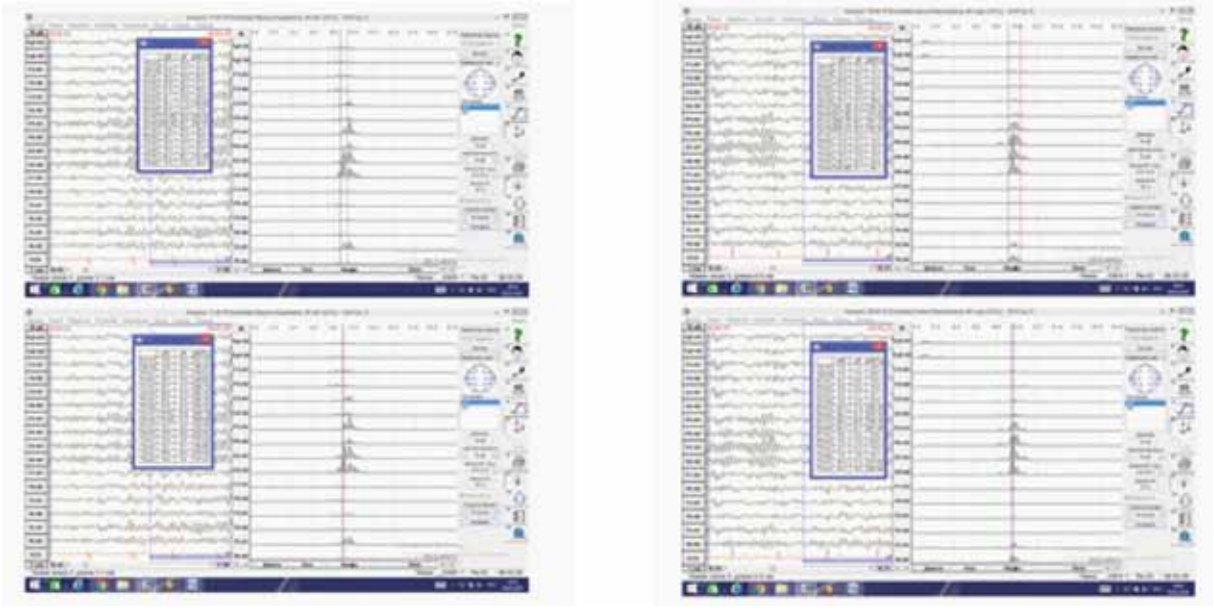
## CONCLUSION

Our study shows that the use of manual tapping according to the author's technique allows reducing the severity of alpha rhythm synchronization and leads to a shift in the peak frequency of the alpha rhythm in the occipital leads towards the alpha2-subfrequency range and an increase in its maximum power in healthy volunteers. Taking into account the theory of hypersynchronization of cortical rhythmics (neural networks) and bioelectrical activity of the brain in general during the development of epileptiform activity on the EEG and epileptic seizures, in particular, the technique we have developed may be promising. as a method of non-drug therapy and (or) prevention of the development of generalized and secondary generalized epileptic seizures at the aura stage or simple focal epileptic seizures. In addition, this wrist-tapping effect indicates clinical use in JME, as it has previously been shown that if selected external frequencies resonate



a b

***Fig. 1.*** *Changes in the characteristics of the alpha rhythm before (A) and after (B) carrying out carpal tapping in the healthy male A. (36 years old): the splitting of the peak of the alpha rhythm in the occipital leads and its transformation into a polypeak is shown*



a b

***Fig. 2.*** *Changes in the characteristics of the alpha rhythm before (A) and after (B) carrying out hand tapping in the a healthy male T. (39 years old): the shift of the peak of the alpha rhythm in the alpha2 subband and an increase in the number of peaks in the occipital leads is shown*

with antiepileptic neurons, an antiepileptic effect can be obtained. However, the confirmation of our hypothesis requires further study with the inclusion of a sample of patients suffering from focal and generalized forms of epilepsy.

*Conflict of interest*

The authors declare no obvious and potential conflicts of interest related to the publication of this article.

*Contribution of authors*

E.A. Narodova — review of publications on the topic of the article, writing the text of the manuscript, designing the article; Shnayder N.A. — concept development, selection of articles for analysis, writing and correction of the manuscript text; Narodova V.V — a review of publications on the topic of the article, the design of the article

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# MICROCIRCULATORY ALTERATIONS IN PATIENTS WITH OROPHARYNGEAL CANCER AFTER RADIATION THERAPY: A POSSIBLE CORRELATION WITH MUCOSITIS?

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**ABSTRACT** — Background: Patients affected by several forms of malignant neoplasms receive chemotherapy (CT) or radiation therapy (RT). These treatments can cause many side effects, such as oral mucositis (OM).
Mucositis is the most frequently occurring early side effect of conservative treatment of patients with malignant tumors in the head and neck, and it is registered in more than 60% of cases. It occurs due to the effect of chemotherapeutic drugs on the cells of the mucous membrane, which causes their death, and to a greater extent, due to the effect of ionizing radiation on the endothelium of the blood vessels and the basal cells of the mucous membrane the submucosa. Objectives: To assess the correlation between the indicators of hemomicrocirculation of the oral mucosa and the intensity of the clinical manifestations of oral mucositis. Materials and Methods: This study included 48 patients who had a morphologically confirmed diagnosis of squamous cell carcinoma and received radiation therapy at National Medical Research Radiological Centre (Moscow, Russia). Results: It was found that, in all the subgroups, the severity index of mucositis National Cancer Institute (NCI) clearly correlated with the indicators of the flow of microcirculation through the study area at point A (r = -0.85, -0.99 and -0.77). At point A, blood perfusion in the study of hemomicrocirculation in all the subgroups 18–44 g in Ia, 45–59 g and 60–74 g in Ic was the opposite of the value of the severity of mucositis. A strong negative correlation was found between the severity of mucositis and the perfusion index at point B in subgroup Ia : (r = -0.99) along with, a moderate inverse correlation in subgroups Ib (r = -0.69) and Ic (r = -0.36). At point B, a strong inverse correlation was found in subgroups Ib and Ic (r = -0.72 and -0.65, respectively), and a moderate inverse correlation was found in subgroup Ia — NCI where r = -0.32. Conclusions: There is a negative correlation between the indicators of hemomicrocirculation of the oral mucosa and the severity of oral mucositis. It was found that the higher the lesions of the microvasculature, the lower the intensity of mucositis. These data have important prognostic value and make it possible to recommend the determination of hemomicrocirculation as a screening test.

**KEYWORDS** — mucositis, oral cavity, radiation therapy, microcirculation, correlation.

## BACKGROUND

Patients affected by several forms of malignant neoplasms receive chemotherapy (CT) or radiation therapy (RT). These treatments can cause many side effects, such as oral mucositis (OM).

Mucositis is the most frequent early side effect of conservative treatment of patients with malignant tumors in the head and neck, and it is registered in more than 60% of cases. It occurs due to the effect of chemotherapeutic drugs on the cells of the mucous membrane, which causes their death, and to a greater extent, due to the effect of the ionizing radiation on the endothelium of the blood vessels and basal cells of the mucous membrane, the submucosa.

The pathogenesis of OM provides for a vascular phase at the onset of lesions in the oral cavity, but the literature lacks data on the correlation between OM and the changes in microcirculation in the oral cavity. Avanesov A.M. described the dependence of the hemomicrocirculation indices in the oral mucosa in patients diagnosed with squamous cell carcinoma of the oropharyngeal region on the severity of clinical manifestations of mucositis (2018) (5). Other researchers have assessed the perfusion of the oral mucosa by monitoring and quantifying the density of the capillaries before and after the administration of CT and RT in cancer patients (Gvozdikova E.N. 2017) (8). This study shows that there is a relationship between the state of hemomicrocirculation of the oral mucosa and the intensity of oral mucositis.

*Objective:*

to assess the correlation between the indicators of hemomicrocirculation of the oral mucosa and the intensity of the clinical manifestations of oral mucositis.

## MATERIALS AND METHODS

This study included 48 patients who had a morphologically confirmed diagnosis of squamous cell carcinoma and received radiation therapy. All the patients underwent treatment at the Department of Radiotherapy, National Medical Research Radiological Centre (NMRRC), Russian Federation. All the patients were divided into 3 age groups:

Ia — patients from 18 to 44 years of age
Ib — patients from 45 to 59 years of age
Ic — patients from 60 to 74 years of age.

The average age of the patients was 53 years. The patients comprised 32 men and 16 women. Ib was the largest group, and 28%.

Localization of malignant neoplasms was dominated by lesions of the tongue (13%), the bottom of the oral cavity (8.3%), the upper jaw (7%), the oropharynx (45.8%), the nasopharynx (7%), and the tumors of other localizations (lesions of the lip, cheek, and alveolar ridge of the lower jaw) accounted for 18.9%.

The division into subgroups was based on the voluntary consent of the patients to follow the recommendations of the dentist and to use the prescribed treatment regimens for the prevention and treatment of mucositis in the presence of radiation. All the patients of the subgroups Ia, Ib, and Ic received daily local dental treatment according to the following guidelines:

1. During irradiation, patients should avoid consuming hot, spicy, solid food and alcohol and should stop smoking.

2. They should brush their teeth using a soft-bristled brush 2 times a day (morning and evening). Patients are advised to use a paste without irritating substances, such as toothpastes manufactured for babies. The use of dental floss (dental floss) is prohibited.

3. They should rinse their mouth with broths of sage, chamomile, and long-acting drug Tonsinal 6–8 times a day. After eating, they should rinse their mouth with antiseptic solutions of Chlorhexidine (0.05%) or Miramistin (0.01%). After rinsing, it is recommended to apply castor oil to the mucous membrane of the mouth for moisturization. The use of alcohol containing rinses for the prevention and treatment of mucositis is contraindicated.

4. They should moisturize their lips with petroleum jelly and hygienic lipstick 3–4 times a day.

5. The use of removable dentures is not recommended since it can lead to excessive irritation of the mucous membrane and increase the pain syndrome. The use of partial or complete dentures should be minimized or avoided.

6. They should ensure daily applications of CM-1 plates and Pharmadont 1–2 plates (which were prescribed depending on the severity of clinical symptoms) 2–3 times a day.

7. The control of the level of individual hygiene and measurement of hemomicrocirculation for each stage of radiation therapy should be as follows: before irradiation, 0–20 Gy, 22–40 Gy, 42Gy, and more, and after irradiation, at the dentist.

To determine the parameters of microcirculation, 48 patients of the 1st group were checked before and after undergoing radiation therapy and administered with doses at the rate of 0–20 Gy, 22–40 Gy, 42 Gy and more during its various stages. Measurements were taken at four randomly selected points: A — mucous membrane of the alveolar gums in the area of teeth 11 and 21; B — the mucous membrane of the lower lip in the projection of the site of the infestation; C — the mucous membrane of the cheek in the projection of teeth 16 and 17; D — the mucous membrane at the bottom of the oral cavity in the projected area where the tongue was attached with the help of the Apparatus Lacc M device (2nd version) (refer to Fig. 1) in the dental office of the NMRRC.


***Fig. 1.*** *Apparatus Lacc M (2nd version)*

The apparatus Lacc-M light guide analyzer ensures the delivery of probe radiation from the laser to the research area and its transport to the photodetectors of radiation reflected from the tissue containing silica in serum.

While interacting with tissue, the reflected signal contains a component produced by the reflection from the moving red blood cells, which is proportional to the speed of movement (Doppler effect). The amplitude of the signals in the device is formed by

all erythrocytes located in the probing region, which move at different speeds and with different numbers and are distributed in the arterioles, capillaries, venules, and arteriovenular anastomoses. At the output of the LACC 02, a signal is formed, which is an indicator of microcirculation PM):

$$PM = Ner \times Vcr$$

where Ner is the number of erythrocytes in the probe volume, Vcr is the average rate of erythrocytes.

The state of microcirculation was assessed according to several parameters with the help of Laser Doppler flowmetry (LDF)

M — the average value of the microcirculation indicator, i.e., the average blood flow in a given time interval in perfusion units, which characterizes the tissue hemomicroperfy;

$\sigma$ — the root mean square deviation of the amplitude of the oscillation of the blood flow, and it reflects, among other things, the elasticity of the wall of the blood vessel

Kv — the coefficient of variation, i.e., the ratio of $\sigma$ to M, and it is the most objective parameter that allows the assessment of the state of microcirculation as a whole.

Given the small number of observations, the statistical processing was carried out by using the sign test (a non-parametric method).

Ethical approval for our study was obtained (No. 0318) from the Ethics Committee of the Institute of Medicine, Peoples' Friendship University of Russia, Moscow, Russia.

## RESULTS AND DISCUSSION

The results are presented in Tables 1, 2, 3, and 4.

It was found that, in all the subgroups, the severity index of mucositis NCI clearly correlated with the indicators of the flow of microcirculation through the study area at point A: NCI — subgroup Ia (r = -0.85); NCI — subgroup Ib (r = -0.99) NCI — subgroup Ic (r = - 0.77). The (Pearson) correlation coefficient was close to -1, which means that there was a strong negative correlation between the variables. In other words, at point A, blood perfusion in the study of hemomicrocirculation in all the subgroups 18–44 in Ia, 45–59 in Ib and 60–74 in Ic was opposite to the value of the severity of mucositis. It was found that the severity of mucositis will decrease with the increase in blood perfusion in the study of microcirculation.

The results of the ratio of the severity of mucositis to the hemomicrocirculation index at point B are presented in Table 2.

A strong negative correlation was found between the severity of mucositis and the perfusion index at point B in subgroup Ia (r = -0.99), and a moderate inverse correlation was found in subgroups Ib (r = -0.69) and Ic (r = -0.36).

Table 3 shows that at point C, a strong inverse correlation was found between the severity of mucositis and the hemomicrocirculation indices in the subgroups Ib and Ic (Ib — NCI: r = -0.72, Ic — NCI: r = -0.65) and a moderate inverse correlation was found in subgroup Ia (Ia — NCI: r = - 0.32). This indicates that at point B, the increase in the severity of mucositis, which we found in the patients in subgroup Ia, was higher than that in the rest even though the microcirculation indices in all the subgroups tended to recover. The frequency and severity of oral mucositis in subgroup Ia changed more slowly. This suggests that other factors also affected the microcirculation indices in the buccal mucosa in the projection of teeth 16 and 17 in subgroup Ia.

As can be observed in Table 4, the correlation coefficient for all the subgroups has negative values. However, in group Ia, the intermediate values were close to 0 (r = -0.04), which indicates a weak correlation between the severity of mucositis and the indicators of hemomicrocirculation in the mucous membrane of the floor of the mouth in the projection of the frenulum attachment site language. However, in subgroup Ib — NCI: r = -0.5 and Ic — NCI: r = -0.73, there was a strong negative correlation. This indicates a strong relationship between the indicators of hemomicrocirculation of the blood vessels and the intensity of clinical manifestations of oral mucositis.

## CONCLUSION

The comparative analysis of the effectiveness of local treatment, depending on the age of the patients, demonstrated a higher efficiency of dental support in the age groups of 18–44 years and 45–59 years and a lower efficiency in the age group of 60–74 years.

It was found that radiation therapy causes changes in the hemomicrocirculation of the tissues of the oral mucosa. The tissue perfusion rates were reduced by up to 50% depending on the age group. After dental support, there was a 12.4% decrease in subgroup Ia, a 24.5% decrease in group Ib and a 33.4% decrease in subgroup Ic.

There is a negative correlation between the indicators of hemomicrocirculation of the oral mucosa and the severity of oral mucositis. The higher the lesions of the microvasculature, the lower is the intensity of mucositis. These data have important prognostic value and make it possible to recommend the determination of hemomicrocirculation as a screening test.

***Table 1.*** *Hemomicrocirculation at point A versus NCI severity of oral mucositis*

| Measurement point | Subgroups | Before radiation therapy | 0–20 Gy | 22–40 Gy | 42 Gy and more | After treatment | Correlation coefficient (r) |
|---|---|---|---|---|---|---|---|
| Point A | Ia (18–44 years) | 44.70 | 32.28 | 34.19 | 31.08 | 34.03 | -0.85 |
| | NCI | 0 | 0.89 | 0.67 | 1.11 | 0.22 | |
| | Ib (45–59 years) | 35.78 | 23.94 | 25.13 | 23.66 | 26.23 | -0.99 |
| | NCI | 0 | 0.29 | 0.21 | 0.36 | 0.07 | |
| | Ic (60–74 years) | 24.55 | 13.94 | 16.29 | 12.95 | 15.61 | -0.77 |
| | NCI | 0 | 0.73 | 0.55 | 0.91 | 0.18 | |

***Table 2.*** *Hemomicrocirculation at point B versus NCI severity of oral mucositis*

| Measurement point | Subgroups | Before radiation therapy | 0–20 Gy | 22–40 Gy | 42 Gy and more | After treatment | Correlation coefficient (r) |
|---|---|---|---|---|---|---|---|
| Point B | Ia (18–44 years) | 19.51 | 14.82 | 15.72 | 13.87 | 16.73 | -0.99 |
| | NCI | 0 | 0.89 | 0.67 | 1.11 | 0.22 | |
| | Ib (45–59 years) | 20.48 | 10.48 | 16.14 | 11.77 | 14.95 | -0.70 |
| | NCI | 0 | 0.29 | 0.21 | 0.36 | 0.07 | |
| | Ic (60–74 years) | 18.11 | 5.45 | 9.08 | 6.33 | 7.02 | -0.36 |
| | NCI | 0 | 0.73 | 0.55 | 0.91 | 0.18 | |

***Table 3.*** *Hemomicrocirculation at point C versus NCI severity of oral mucositis*

| Measurement point | Subgroups | Before radiation therapy | 0–20 Gy | 22–40 Gy | 42 Gy and more | After treatment | Correlation coefficient (r) |
|---|---|---|---|---|---|---|---|
| Point C | Ia (18–44 years) | 20.07 | 14.02 | 15.97 | 14.16 | 17.29 | -0.32 |
| | NCI | 0 | 1.78 | 1.33 | 1.44 | 1.56 | |
| | Ib (45–59 years) | 18.32 | 9.49 | 16.39 | 10.74 | 13.82 | -0.72 |
| | NCI | 0 | 0.57 | 0.43 | 0.46 | 0.5 | |
| | Ic (60–74 years) | 18.11 | 10.53 | 17.42 | 10.18 | 13.09 | -0.65 |
| | NCI | 0 | 1.45 | 1.09 | 1.18 | 1.27 | |

***Table 4.*** *G-point hemomicrocirculation index versus NCI severity of oral mucositis*

| Measurement point | Subgroups | Before radiation therapy | 0–20 Gy | 22–40 Gy | 42 Gy and more | After treatment | Correlation coefficient (r) |
|---|---|---|---|---|---|---|---|
| Point D | Ia (18–44 years) | 16.50 | 12.42 | 13.88 | 11.80 | 14.44 | -0.04 |
| | NCI | 0 | 1.56 | 1.11 | 1.44 | 1.67 | |
| | Ib (45–59 years) | 14.87 | 5.91 | 12.39 | 8.26 | 10.76 | -0.50 |
| | NCI | 0 | 0.5 | 0.36 | 0.46 | 0.54 | |
| | Ic (60–74 years) | 11.63 | 5.95 | 9.52 | 7.00 | 7.74 | -0.73 |
| | NCI | 0 | 1.27 | 0.91 | 1.18 | 1.36 | |

## DISCUSSION OF THE RESULTS

There is no gold standard for cancer treatment. Cancer treatment usually includes surgery, RT, CT, or a combination of these on an individual basis. The administration of these agents can determine the onset of oral lesions such as oral stomatomucositis (4). The risk factors leading to the development of oral post-radiation mucositis are versatile: chronic alcohol consumption, cigarette smoking, low body mass index (BMI < 18.5) as well as concomitant diseases, such as diabetes mellitus, hypertension, and atherosclerosis.

This study showed that the PM microcirculation index in patients varies unevenly throughout the entire stage of radiation and/or chemotherapy. An increase in microcirculation indices reached the maximum point at a dose of 20–28 Gy, which corresponded, on average, to the first clinical manifestations of oral mucositis in the patients (hyperemia and swelling of the mucous membrane), followed by a decrease in the indicator to values that were 49.6% less than the initial values.

In our study, we noted that there is a negative correlation between the indicators of hemomicrocirculation of the oral mucosa and the severity of oral mucositis. The higher the lesions of the microvasculature, the lower is the intensity of mucositis. These data have important prognostic value and make it possible to recommend the determination of hemomicrocirculation as a screening test.

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# DENTAL ORTHOPEDIC REHABILITATION IN PATIENTS WITH PROBLEMS RELATED TO TYPE 2 DIABETES. LITERATURE REVIEW

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**ABSTRACT** — The paper discusses modern approaches to restoration of dentition defects in patients with type 2 diabetes mellitus (DM). Endocrinopathy severely affects the status of the teeth periodontium, the oral mucosa and the jaw bone tissue and poses a challenge to the denture design. This accounts for the techniques employed to prepare supporting teeth for prosthetics and the choice of construction materials which should ensure optimal functional capacity, biocompatibility and aesthetics. Besides, our article reveals systematized specifics of prosthetic treatment using dental implants in patients suffering from diabetes.

**KEYWORDS** — literature review, secondary adentia, diabetes mellitus, dental prosthetics.

## BACKGROUND

Tooth loss results in a worse quality of life caused by deterioration in chewing efficiency and aesthetic dissatisfaction. Prosthetics of dentition defects in individuals with type 2 diabetes enables not only to restore the dentition function and aesthetics, yet it also produces a positive effect on the course of the major disease through improving the glycemia level [1]. This means that prosthetics of missing teeth appears as an important step in comprehensive rehabilitation for patients with type 2 diabetes. During that, it is to be noted that dentists are extremely cautious when it comes to discussing truly successful prosthetics for diabetic patients, and in view of that, we decided to focus on modern issues of orthopedic dental treatment offered to patients with type 2 diabetes.

Numerous studies have revealed a high prevalence of tooth loss in diabetic patients [2, 3, 4, 5]. A number of authors have found that along with an increase in the age of patients and their diabetes experience, there is a significant increase in the number of removed teeth within the structure of the CFR-indicator (C-caries, F-filling, R-removed tooth) [6]. Besides, as a study by Yonekura S. suggests that the number of removed teeth correlates with the level of glycated hemoglobin (HbA1c): individuals with poorly controlled DM (HbA1c ≥ 9%) featured a greater number of removed teeth, if compared to patients with well or moderately controlled DM (HbA1c ≤ 9%) [7].

Therefore, due to a higher occurrence and a large number of teeth removed, patients with type 2 diabetes are in an urgent need to restore the dentition and improve their chewing efficiency. R.A. Kerimov, for instance, in his work focusing on dental rehabilitation for patients with type 2 diabetes, claims that the need for prosthetics was identified in 95.24% of the patients[8]. Rumyantseva E.V. et al. claim that a study involving 76 patients with type 2 diabetes, showed that 78.3% of them falling within the age group of 35–44) needed prosthetics; within the group of those aged 45–64, that need was identified in 95.4% of patients, whereas 100% of patients aged 65–74 needed prosthetics [9].

The high need for orthopedic treatment in patients with type 2 diabetes comes along with great difficulties impeding the process of dental prosthetics due to a number of pathological issues in the oral cavity: periodontal inflammation of the supporting teeth; reduced resistance of the oral cavity capillary vessels (OCCR); progressive atrophy of the alveolar process; paresthesia and perverted taste; the OCCR spilled inflammation, especially in case of candidiasis, and undoubtedly the mucous membrane dryness in the prosthetic bed. Given that, when replacing dental defects in patients with type 2 diabetes, there is a number of requirements dealing with the technique of preparing supporting teeth, dental prosthesis design and materials.

## MODERN APPROACH TO DENTAL DEFECTS PROSTHETICS IN PATIENTS WITH DIABETES

Restoring and maintaining proper oral hygiene is a mandatory step preceding any orthopedic intervention [10, 11].

The preparation of teeth should be done carefully in order to avoid soft tissue injury due to poor wound healing. This also explains why the preparation of supporting teeth should be performed strictly following the requirements of asepsis and antiseptics, whereas after the preparation of hard tissues, the sharp edges of the teeth should be smoothened, with their surfaces treated with a respective polish. When compensating for partial loss of teeth with removable plate prostheses, impressions should be obtained using alginates. To create supporting structures of such prostheses, we recommend obtaining casts and employing the sandwich method [12].

When carrying out tooth replacement in patients with type 2 diabetes, it is a good idea to expand the indications for using fixed prostheses, which have basically no pressure on the mucous membrane while having minimal contact with it. However, it is important to take into account the existing periodontopathies that complicate the process of installing fixed prosthetics due to constant inflammation of the gingival papillae, and even a slight touch of the crown can cause vascular injury, exudation, and subsequent inflammation. Given the above, the preparation of supporting teeth should be performed with a bevel above the gum level and without a ledge, since the latter can concentrate stress on the periodontal area of the already weakened teeth [12].

The bridge-like prosthesis body cannot join the alveolar process mucous membrane in order to avoid its mechanical injury. The bridge structures should be well polished (quality degree — 9–10) with no sharp and protruding elements [13].

Dental mobility against periodontitis in patients with type 2 diabetes often complicates prosthetics of dental defects. In such cases, it appears rational to manufacture dental splint structures of dentures that would compensate for the missing dentition at the same time immobilizing the mobile teeth [13, 14]. V.N. Sukholitsky proposed using plastic-lined non-removable solid splinting dentures. A special point featured by the proposed prosthesis design is the frame with a metal garland at the neck area of the dental crowns, as well as the intermediate part with vestibular and oral surfaces. This design allows reaching optimal distribution of loads on the metal part of the frame, while this also prevents chips of the facing material, as well as improves the hygiene in the area of structures and the oral cavity, which is due to the absence of facing material (plastic) at the neck area [15].

Diabetic patients have an alveolar process bone metabolism that is impaired, which, in turn, affects the reparative and regenerative processes [16–21]. Any prosthesis can aggravate the situation, causing rapid progression of bone atrophy. In case of type 2 diabetes, the oral mucosa develops serious changes in microvessels as well as hemodynamic disorders, significant dystrophy and oral mucosa' epithelial cells atrophy. As for removable prostheses, the basis exerts pressure on the oral mucosa and, respectively, on the capillaries, which makes the hemodynamics disturbance even worse. This, in turn, will lead to disturbed trophism in the mucous membrane, and will entail complications in the subprosthetic bed. This means that in case of dealing with removable dentures indicated for people with type 2 diabetes, a better choice would be clasp and plate prostheses with supporting and retaining elements (clasps, attachments, telescopic crowns, beam and magnetic fixation systems), which allow unloading the oral mucosa. Another option in this case is a plate prosthesis with a two-layer base made of plastic with an elastic lining [22, 23].

A number of studies focusing on work with people with diabetes have demonstrated the advantage that metal-base partially removable prostheses over partially removable plate prostheses. The design of metal-base partially removable prostheses allows including splinting elements, which makes it the most acceptable solution for patients with DM. Also, a partially removable prosthesis with a metal base has a smaller triggering effect on the development of clinical symptoms of oral dysbiosis, as well as has a lower traumatic effect, while patients experience less of an issue adapting to it, if compared to a partially removable plate prosthesis [24].

Nearly 50% of all patients with diabetes have oral candidiasis, which is related to intolerance to many structural materials. The complexity of prosthetics is due to the fact that any removable prosthesis creates under itself perfect conditions for fungal microflora growth and reproduction, which triggers the development of oral dysbiosis or acute fungal stomatitis. Given that, when it comes to manufacturing removable prostheses, it is important to choose materials that do not facilitate microbial colonization on their surface. A number of studies focused on comparing microbial colonization on the surface of a conventional acrylic prosthesis, which was matched against polyamide and nylon flexible thermoplastic polymer prostheses, when used for prosthetics in patients with type 2 diabetes.

A flexible thermoplastic polymeric prosthesis made of nylon is a more predictable treatment method due to its lower microbial colonization and relatively healthier biological tissue response, apart

from a better aesthetic appearance [25, 26]. Silver ions help improve the activity of saliva enzymes, so this allows recommending the manufacture of dental prostheses from silver and palladium-based alloys [27]. To reduce microbial contamination of dentures, a number of authors suggest treating them with a 4% solution of chlorhexidine gluconate [28], as well as disinfecting them in an Ozon-Stom device [29] or in a microwave oven [30]. Applications of acid-soluble chitosan to the mucosa at the prosthetic bed prove effective for restoring the microbiocenosis of the oral mucosa in patients with diabetes who wear removable prostheses [24]. Good glycemic control ensures a level of biofilm development on the surface of the prosthesis, which is similar to that in healthy people [31].

A modern alternative in orthopedic dental rehabilitation of diabetics is prosthetics on implants. Earlier, there was a common idea that the indication for dental implantation in people with type 2 diabetes implied strict control of glycemia, whereas poor glycemic control was a contraindicating factor to prosthetics on implants. However, recent studies allow expanding the indications for implantation in people with type 2 diabetes. C.C. Eskow and T.W. Oates point at a high survival rate of dental implants after one year (98.6%) and 2 years (96.6%) in patients with poorly controlled diabetes (8.0% ≤ HbA1c ≤ 12.0%) [32]. In an earlier study, Oates T.W. et al. estimated the survival rate of implants for 1 year, after it was exposed to a load in patients with poorly controlled type 2 diabetes, at 95.0%, which is comparable to similar indicators for implantation of somatically healthy patients and individuals with well-controlled DM.

However, the negative impact of poor glycemic control on early bone healing and primary implant stability was noted [33]. These data confirm the possibility of wider use of implantation therapy in patients with type 2 diabetes and poor glycemic control. However, in case of good glycemic control, the survival rate of implants 5 years after the installation, and the bone loss around the implant in diabetics, were comparable to the same indicators in individuals without chronic pathology, which serves another proof to the importance of glycemic control in the dental rehabilitation of patients with type 2 diabetes [34].

For people with DM, a protocol of delayed implant introduction (installation 4–6 months following the tooth extraction) is recommended, which is due to a lower level of bone tissue loss around the implant, if compared to immediate and early (6–8 weeks after the tooth extraction) installation techniques. There was no difference in the clinical and radiological status of implants installed in diabetics with immediate and normal loading [35].

Recently, there has been success demonstrated in immediate implantation and prosthetic rehabilitation employing the All-in-Four method, when working with patients with type 2 diabetes. In R.I. Juncar's study, implants demonstrated good osseointegration and stability 6 months after the installation in individuals with diabetes; however, the authors emphasize the importance of maintaining proper oral hygiene and glycemic control for better postoperative recovery [36].

Prosthetics on implants helps reduce the indications for removable prosthetics, avoid overloading the supporting teeth with excessive occlusal stress through non-removable prosthetics, and improve the patients' adaptation to the dentures. In view of that, prosthetics on implants appear an acceptable treatment for patients with diabetes, with a good risk/benefit ratio [37].

When installing prostheses in patients with type 2 diabetes, the issue of the biocompatibility of materials used to make permanent prostheses is extremely relevant, since this is what comes into close and long-term contact with the gum. P. Saravanakumar's study focused on evaluating the effect that various crown materials (metal, ceramics, and zirconium dioxide) work on the content of beta-interleukin-1 (IL-1β) in the gingival fluid of the supporting teeth in somatically healthy patients. IL-1β is a powerful inflammatory cytokine and is a marker indicating acute inflammation in tissues, including periodontal teeth. A three-month long observation of the effect that crowns, made of metal, metal-free ceramics and zirconium dioxide, have on the marginal gum, revealed that crowns made of zirconium dioxide feature the least inflammation in the gums [38]. The obtained data suggest that dentition defects prosthetics in diabetic patients with crowns made of zirconium dioxide is a more preferable option. However, literature does not offer enough coverage of this issue, and requires additional investigation, since prosthetics in patients with type 2 diabetes is usually done along with inflammation of periodontal teeth on.

## CONCLUSION

1. When restoring the dentition in people suffering from type 2 diabetes, it is recommended to expand the indications for prosthetics with fixed structures, including those based on dental implants, as well as manufacturing prostheses from bioinert materials such as zirconium dioxide.

2. In case of indications for removable structures in persons with type 2 diabetes, the preferred option includes splinting and plate prostheses with supporting and retaining elements or a two-layer basis in order to unload the oral mucosa.

3. When selecting the design of removable dentures, a better choice would be prostheses made of materials that feature lower microbial colonization on their surface, for example, those made of thermoplastic polymeric nylon or silver- and palladium-based alloys.

4. The key to successful prosthetics of dental defects in diabetics is good glycemic control and maintaining a good level of oral hygiene.

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# PERIODONTIUM INFLAMMATORY DISEASES IN ORTHODONTIC TREATMENT WITH FIXED DENTURES. LITERATURE REVIEW

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**ABSTRACT** — The article offers a review of research literature containing data on treating the occlusion pathology with fixed orthodontic equipment, as well as details regarding potential complications affecting periodontium tissues, with a clinical case offered as an example. Statistical reviews suggest high prevalence of this pathology and its significance in the structure of dental diseases. Analysis suggests that there is a need to study the available research and knowledge accumulated in the field of dentistry, which may contribute to the development and implementation of the most effective preventive measures.

**KEYWORDS** — periodontal disease, occlusion anomaly, dental anomalies prevalence, hygiene indices.

Periodontal tissue diseases are common among adult population. Periodontal tissue damage may be due to genetic predisposition, other diseases such as diabetes or issues affecting the cardiovascular, gastrointestinal or respiratory system; disturbed hormonal status; chronic intoxication, etc., as well as can be attributed to local factors such as poor oral hygiene, prominent filling edges, orthopedic structures that do not meet the manufacturing and fixing requirements, eating foods rich in carbohydrates, or things like malocclusion, abnormal position of teeth, which are to be found in most people, as well as wearing orthodontic devices. In this article, we will focus on periodontal diseases that occur at the stage of orthodontic treatment in adult patients using fixed orthodontic equipment and have no concurrent diseases, as well as we will take a look at methods of prevention and treatment for such issues.

Orthodontic treatment has both indications and contraindications. Absolute contraindications for treatment with a bracket system include immune system diseases; malignant tumors and some oncological diseases; cardiovascular system issues; blood diseases; certain diseases of the bone system involving the healing process; endocrine system issues; tuberculosis; neuro-mental diseases; HIV; STDs; lack of numerous teeth. This issue has been covered in a work focusing on studying periodontal diseases [8]. Some of the relative contraindications include poor oral hygiene, as claimed by Yu.M. Glukhova, A.I. Kiryutin [1], and periodontal diseases, which tend to affect more and more of younger patients in Russia, while the share of these aged 15–19 years is 55–89%, and in the adult population this figure goes up to 99%. A number of authors [2] point at the patient's unwillingness to undergo treatment. However, be that one way or another, all these factors can be eliminated.

One of the methods employed to prevent the aggravation of mild periodontitis into its generalized chronic type is orthodontic treatment. Orthodontic treatment can only be performed in case of a healthy periodontium or when periodontal diseases is in remission (lasting for at least 2 years). Our major task is to find out what periodontal diseases occur during orthodontic treatment, and whether there is any connection with a specific maxillofacial pathology (MFP).

When keeping the patient's medical record, the orthodontist collects carefully the patient's history of life and illness because it is important to identify and evaluate the patient's health status, detect concomitant diseases, which may appear as a contraindication to orthodontic dentures installation. Such diseases have already been mentioned in this article [3–6].

After making sure there are no contraindications, orthodontic treatment can be started, which in Russia is carried out in adult patients with removable and non-removable dentures that being the most common type of treatment. The type of technique often depends on the gum phenotype,

the mechanics of treatment, its duration, and most importantly, the type of MFP. According to [7], the dental anomalies prevalence in the adult population is 30–55%.

In this country, the most common type of occlusion is neutral with a close position of the teeth; the most common pathology is distal occlusion; the second most common is deep occlusion; there are fewer patients featuring mesial, open and cross occlusions. Similar data were obtained by [8], where the authors found that the share of neutral occlusion accounts for 43.11 % of those who were identified as in need of dental care; 25.52% had a distal occlusion, whereas another 4.99% had a mesial occlusion.

The most common type of occlusal ratio disturbance is a combination of distal and deep, which, under the effect caused by plaque microorganisms, serves an obstruction to proper individual cleaning of the oral cavity from plaque. This situation is aggravated by a bracket system installed on the outer or inner surface of the teeth.

All patients with healthy periodontium feature changes in the blood microcirculation parameters in the gums (by 79% in the first 7 days from the moment elastics were applied to non-removable orthodontic equipment). This conclusion was offered by K. A. Evnevich [9], who confirmed his study outcomes with the LAKK-01 device at the square arcs stage.

There is research data available indicating the appearance of generalized catarrhal gingivitis signs within 2 months after the installation of non-removable orthodontic equipment in all patients [10, 11].

Also, a factor triggering periodontal diseases is nocturnal and diurnal jaw compression, which is most often observed in patients with a deep bite [12]. An important factor that has an impact on the periodontal status is smoking [13]. Nicotine changes the subgingival microflora composition, suppresses protective mechanisms that promote the elimination of periodontal pathogens. Patients report bleeding gums, bad breath, changed gum color and pain when cleaning. In 80% of cases, following a professional oral hygiene procedure, patients complain of tooth sensitivity [14].

The next most common MFP in the sagittal plane is mesial occlusion, which is often accompanied by multiple recessions of the gingival margin, especially at the central lower incisors, the reason behind that being their inverse ratio and traumatic occlusion [15].

Regardless of the location, soft plaque turns into calcified deposit, which exacerbates the degree of gingivitis. Given the periodical frequency of appointments that orthodontic patients follow (once a month, once every 2 months), gingivitis, if other conditions are in place, can turn into a more severe stage — periodontitis, which features symptoms like bleeding gums, destructed alveolar partitions, development of dental-gingival pockets, purulent discharge, abnormal tooth mobility, as well as appearance of bacteria like Streptococcus mutans, S. sanguis and S. oralis, Porphyromonas gingivalis, Treponema denticola, S. sobrinus, Streptococcus salivarius, and S. macacae, which, together with orthodontic displacement, can change the bone tissue structure in the alveolar process of the upper and lower jaw, as well as irreversible tooth mobility, and make the gingival papilla disappear, which in modern dentistry can only be restored with hyaluronic acid, except cases where surgical reconstruction is to be employed, as stated by [16–19].

The dentist will evaluate the patient's periodontal status from a clinical stance, which, according to a number of authors [20], typically has the following characteristics: a healthy periodontium when probing the gingival groove; no bleeding from the gingival papilla; the groove depth in the formed periodontal front teeth — 0.5 mm, in the side teeth — up to 3.5 mm. In the event the parameters do not fall within the specified normal range, additional evaluation is to be performed: periodontal status evaluation based on periodontal indices; computer diagnostics for examining periodontal tissues; X-ray examination; CBCT; measurement of the periodontal pocket depth; biomicroscopy; polarography; laser tissue oximetry; laboratory research methods, etc [21–24].

In conclusion, it is to be emphasized once again the importance of collecting data regarding the patient's life and illness, complaints, basic and additional methods of examination prior to the orthodontic treatment, since this information is important when it comes to selecting the right tool to correct malocclusion and ensure excellent outcome, keeping the patient's periodontium healthy. To support all of the above, here we offer a clinical case description.

## CLINICAL CASE REPORT

Patient M., 25 y.o., came to the clinic with complaints of poor dental aesthetics. Following the complaints, anamnesis of life (general diseases and allergy — negative) and diseases (previous orthodontic treatment – not performed), objective and additional research methods (CBCT, side TRG), the diagnosis was set: distal deep incisor occlusion; narrowing and shortening of both dental arches; abnormal position of teeth on both jaws; tooth

erasability on both jaws; displaced central line on the lower jaw to the left (by 2.5 mm). A treatment plan was developed, which included oral sanitation and professional hygiene, followed with installation of fixed orthodontic equipment designed as a self-ligating bracket system, after correcting the teeth position and the bite; installation of retention devices and rational prosthetics aimed at raising the bite and eliminating its deep overlap. For the entire period of treatment, the patient was given a memo with recommendations for dental, and for the bracket system care.

At the time the patient came to seek assistance and prior to the installation of the bracket system, the patient's periodontal status (Fig. 1) was evaluated based on 4 indices:

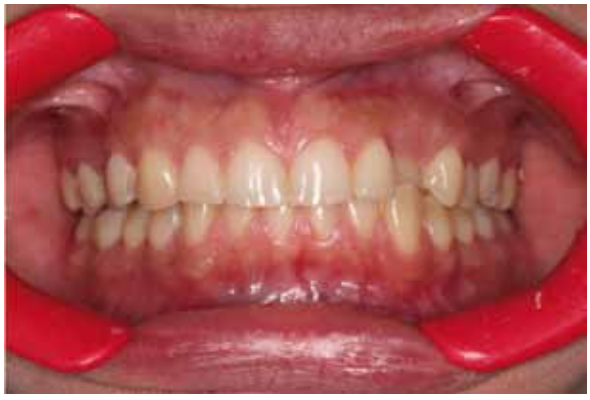

***Fig. 1.*** *Patient M., 25 y.o. The periodontium status at the moment the patient came to seek help prior to installing the bracket system*

1. The Green-Vermillion hygiene index — 0.83, which indicates a good hygiene value.
2. The gingivitis index PMA = 0, which is indicative of the absence of gingivitis.
3. The periodontal index CPITN = 0.0: a low level of periodontal diseases intensity.
4. The gum recession index points at no recession.

At the orthodontic treatment stage, the patient underwent repeated professional dental cleaning (once every 3–4 months), yet the individual hygiene was assessed as poor. The patient was told repeatedly about the level of dental cleaning; after 15 months the periodontal status was assessed through the same indices, which revealed a deterioration in the indicators (Fig. 2). The study showed that:

1. The Green-Vermillion hygiene index was 2, which means a satisfactory hygiene index.

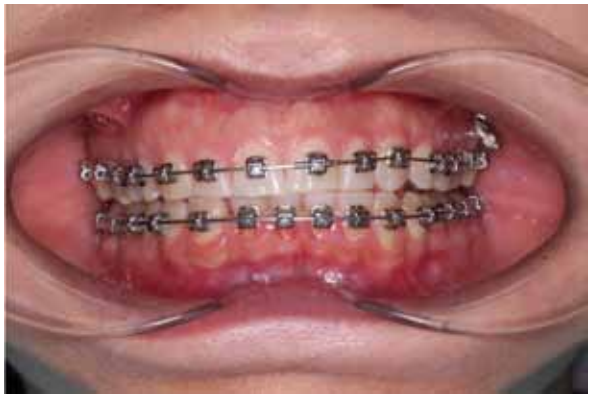

***Fig. 2.*** *Patient M., 26 y.o. The periodontium status after 15 months of orthodontic treatment*

2. The gingivitis index PMA = 55%, which indicates the average degree of gingivitis.
3. The periodontal index CPITN = 0.6 — corresponds to the average intensity of periodontal diseases.
4. The gum recession index = 1.0, which means a mild degree of gum recession.

The data offered by respective literature and clinical research suggest that oral hygiene in patients with fixed orthodontic appliances may get worse during orthodontic treatment and result in periodontal diseases, subsequently causing the need for premature removal of the bracket system.

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# EFFICACY OF A PLANT-BASED DENTAL GEL FOR CHRONIC SIMPLE MARGINAL GINGIVITIS: A CLINICAL TRIAL

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**ABSTRACT** — High prevalence of inflammatory periodontal diseases in young people along with unfavourable outcomes and long-term consequences cause great damage to health, thereby determining the importance and urgency of this problem in modern dentistry. Herein, we introduced a gel based on sanguiritrin, medicinal extracts of calendula and yarrow, and evaluated its effectiveness in the treatment of chronic simple marginal gingivitis in the clinical study involving 45 patients aged 18 to 23 years (26 females and 19 males). In the treatment group (23 patients), after professional oral hygiene and correction of hygiene skills, the dental gel based on sanguiritrin, medicinal extracts of calendula and yarrow was applied as part of complex therapy, while in the control group (22 patients) the *Cholisal* gel was used. The data obtained from hygienic and periodontal indices following the treatment (OHI-S — 0.39±0.03; PBI — 1.12±0.06; PMA — 16.4±1.31%) showed clinical anti-inflammatory efficacy of the introduced dental gel in the treatment of gingivitis, while positive indices dynamics of the dental status (OHI-S — 0.64±0.03; PBI — 0.42±0.03; PMA — 9.92±1.42%) evaluated in 3rd, 6th and 12th months after the therapy indicated of the stability of obtained results.

**KEYWORDS** — gingivitis, herbal medicine, professional oral hygiene, personal oral hygiene, dental gel, biofilm, sanguiritrin, yarrow, calendula.

## INTRODUCTION

Inflammatory periodontal diseases represent multifactorial infectious pathology caused by the body's immune reaction in response to exposure to the oral biofilm [9, 10, 12]. High prevalence of inflammatory periodontal diseases in young people [1, 3, 7], low level of oral hygiene despite a wide selection of constantly improving hygiene products [8] along with the instability of treatment results [5], adverse outcomes and long-term consequences affecting the health determine significance and urgency of this problem in the modern dentistry and healthcare system [4, 6, 11, 12].

Nowadays there is a promising technique of development and usage of medicinal products made from plant materials [1, 3], which are highly effective, easy to use, well tolerated [2, 7]. Herbal medicines have anti-inflammatory, antimicrobial, analgesic, hemostatic and reparative effects, as well as increase the defence properties of the body [1, 2, 3].

The application of herbal medicine is of considerable interest in dental practice [1, 13] namely use of herbal preparations such as yarrow and calendula in the treatment of gingivitis. In this regard, it is also promising to search for new dosage forms and drugs that could increase the effectiveness of treatment, which determined the goal and objectives of the present study.

*The aim of the research* was to study the efficacy of application of dental gel based on sanguiritrin, medicinal extracts of calendula and yarrow in the treatment of chronic simple marginal gingivitis.

## MATERIALS AND METHODS

The group of young people with diagnosed chronic simple marginal gingivitis and dental crowding consisted of 45 people (26 females and 19 males) aged 18 to 23 years. Patients were randomized into two groups.

In the group 1 (treatment group) that included 23 patients with chronic simple marginal gingivitis K05.10, treatment was carried out according to the clinical guidelines (treatment protocols). After oral cavity sanitation, the dental gel of original composition developed by us (patent for invention No. RUS 2621297 dated 04.05.2016 "Dental gel with plant extract for the treatment of periodontal inflammation and oral mucosa") was applied on gums for 20 minutes twice a day for 10 days.

The dental gel contains sanguirithrin and oil extract of calendula flowers and yarrow herb in a 1:1 ratio. Hydroxyethyl cellulose, glycerin, Cremophor RH-40, sodium saccharinate, mint oil and purified water are used as an ointment base. The components are used in the following proportions (wt. %): sanguirithrin — 0.5; hydroxyethyl cellulose — 2.0; glycerin — 3.0; oil

extract from Calendula officinalis flowers and Achillea millefolium herb — 5.0; cremophor — 1.0; sodium saccharinate — 0.5; Peppermint Oil — 0.1; purified water — up to 100.0.

In the group 2 (control group) that included 22 patients, a standard local antibacterial treatment based on 0.06% solution of chlorhexidine bigluconate and dental gel *Cholisal* was applied after oral cavity sanitation. After hygienic cleaning of teeth 2 times a day, patients were recommended to make oral baths with 0.12% chlorhexidine solution for 3–4 minutes within 14 days and then apply the dental gel *Cholisal* to the gingival margin for 15 minutes twice a day for 10 days.

The diagnosis of periodontal diseases was formulated according to ICD-10: K05.1 chronic gingivitis (K05.10 simple marginal), K07.3 anomalies of tooth position (crowding without tooth extraction) and was substantiated by the data of clinical and instrumental studies.

We have developed an algorithm for treatment of patients with chronic simple marginal gingivitis and dental crowding. The complex therapy included the following stages:

1. Assessment and control of oral hygiene by using plaque indicator with subsequent identification with a mirror at the dentist's office and using chewable tablets at home.
2. Motivation and practicing of rational personal oral hygiene including tooth brushing twice a day with therapeutic and prophylactic toothpaste and medium toothbrush, using mouth rinses with extracts of medicinal plants within 10–14 days, flosses, dental irrigator and special dental brushes in cases with crowding. Cleaning of tongue was performed for 1 minute twice a day with a tongue scraper.
3. Professional oral hygiene, antiseptic and antimicrobial treatment of the oral cavity, prevention of biofilm formation on teeth, elimination of hard dental deposits and polishing of teeth surfaces.
4. Oral cavity sanitation (treatment of carious and non-carious defects, restoration of contact points, elimination of traumatic factors).
5. Application of the dental gel based on sanguiritrin, medicinal extracts of calendula and yarrow of the original composition on gingiva for 20 minutes twice a day within 10–14 days in the test group. In the comparison group, dental gel *Cholisal* was applied.
6. Consultation with an orthodontist aimed to eliminate existing anomalies of occlusion and dentition, if necessary, treatment and dynamic observation.
7. Consultation with a dental surgeon aimed to eliminate anomalies in the attachment of maxillary labial, mandibular labial and lingual frenum.
8. In the presence of concomitant somatic pathology, consultation, treatment and follow-up with appropriate somatic specialists.
9. Abandoning bad habits.
10. General therapy aimed for strengthening health condition including multivitamins, macro- and microelements (course of treatment is 1 month).
11. Dispensary observation in the following 3 months after complex individual treatment included examination, control and correction of hygiene once per month, and then — once every 6 months.

For objective clinical effectiveness assessment of proposed methods in the treatment of chronic catarrhal gingivitis, we analysed clinical and index dynamics of periodontal status in patients from the main and comparison groups after 7 days, 1, 3, 6 and 12 months. Statistical processing of the data was performed using standard software packages for applied statistical analysis: Microsoft Excel (Microsoft Corporation) and Statistica 6.0 (StatSoft Inc.).

## RESULTS AND DISCUSSION

During primary examination, the initial values of OHI-S hygiene index in patients of both groups with chronic simple marginal gingivitis and dental crowding did not differ significantly and were 1.96±0.07 and 1.94±0.05 ($p \geq 0.05$) in the main and comparison group, respectively, which indicated of satisfactory level of oral hygiene. When patients were examined 7 days after the beginning of therapy, OHI-S index in the main group was 0.39±0.03, and in the comparison group — 0.4±0.03 ($p \geq 0.05$), which corresponded to a good level of personal hygiene. In our opinion, the obtained results demonstrate the effectiveness of professional oral hygiene procedure such as high-quality professional teeth cleaning, adequacy of selected personal oral hygiene products, patient motivation and hygiene training. In order to optimize personal oral hygiene, additional conversations and trainings on usage of hygiene products were conducted in both groups of patients. During the follow-up examination after 1 month, the values of OHI-S hygiene index were 0.41±0.04 in patients of the main group and 0.43±0.02 ($p<0.05$) in the comparison group which indicated of good level of personal hygiene (OHI-S<1.2 corresponds to good oral hygiene) and absence of possible negative effect of this indicator on the overall result of treatment. At the same time, no statistically significant difference was found between the values of

both groups (p≥0.05) (Table. 1). During the patient examination 3 months after the treatment, values of the hygiene index were 0.61±0.02 in the main group and 0.59±0.03 (p<0.05) in the comparison group, which points to consistently good level of personal hygiene (OHI-S<1.2 corresponds to good oral hygiene) (Table 1). Control examination 6 months after the therapy revealed a general tendency towards increase of OHI-S values. Accordingly, in the main group, the index was 0.42±0.03, and in the comparison group — 0.41±0.03 (p<0.05), which confirms the need for regular professional oral hygiene since there are hard-to-reach areas of tooth surface that remain not properly cleaned even when a patient uses additional hygiene products (flosses, interdental brushes, irrigators, etc.). When patients were examined 1 year after treatment, OHI-S index was 0.64±0.03 and 0.67±0.03 respectively in the test group and comparison group (p<0.05) (Table 1) indicating a good level of personal hygiene (OHI-S<1.2 corresponds to good oral hygiene).

The average value of PBI bleeding index during the primary examination in patients with chronic simple marginal gingivitis and dental crowding was 3.29±0.05 corresponding to severe inflammation in periodontal tissues, and the initial values of this index did not differ significantly (p≥0.05) in patients of both groups (3.30±0.09 — in the treatment group and 3.26±0.03 — in the control group) (Table 2). When patients were examined 7 days after the beginning of therapy, a decrease in the bleeding rate was observed with the values of PBI index 1.12±0.06 and 1.62±0.04 in the treatment and control groups, respectively (Table 2). The obtained data indicates of the clinical efficacy of treatment methods at the initial stages of complex therapy. During the follow-up examination after 1 month, PBI index in patients of the treatment group was 0.76±0.04, while in the control group - 1.22±0.06 (p<0.05) (Table 2). The observed positive dynamics of PBI index in the treatment group provides the evidence for pronounced primary therapeutic effect of the dental gel in the treatment of chronic simple marginal gingivitis. During patient examination 3 months after the treatment, the values of bleeding index in the treatment group of patients with chronic simple marginal gingivitis were 0.55±0.06, while in the control group — 1.14±0.05 (p <0.05). When patients were examined 6 months after the therapy, the values of PBI index were 0.50±0.04 in patients of the treatment group and 1.17±0.06 in patients of the control group (p<0.05) (Table 2) that could be characterized as a stable dynamics of bleeding index. At the same time, there was an insignificant tendency towards decrease of PBI index within the range of statistical error (p≥0.05). During the control examination 1 year after the *primary* course of treatment of chronic simple marginal gingivitis, bleeding index in patients of the main group was 0.42±0.03, while in the comparison group — 1.14±0.05 (p≥0.05) (Table 2). A decrease in the numerical values of PBI index can be attributed to optimization of personal oral hygiene and decrease in the severity of inflammatory processes in periodontal tissues due to the therapy, including the restoration of normal functional state of microvasculature in periodontium.

The identified significant decrease in PBI index in patients of both groups provides an indirect confirmation of the fact that effective conservative therapy conducted at this stage could achieve normalization of the periodontal tissue status, stable remission of chronic simple marginal gingivitis and prevent its transition into periodontitis. It should be noted that patient compliance with recommendations for personal oral hygiene is of great importance along with repeated plaque identification, motivational conversation with the patient and additional training of personal oral hygiene methods in accordance with the characteristics of occlusion. The obtained data show stable dynamics of decrease in PBI bleeding index (p≥0.05). The numerical values of the bleeding index in patients of the treatment group were significantly lower than those in the control group having the same initial data, which indicates of a greater efficacy of the complex treatment with the dental gel of the original composition.

During initial examination of the patients with chronic simple marginal gingivitis, PMA index was 31.8±2.2%, and 31.4±2.1% (p≥0.05) in the treatment and the control groups, respectively (Table 3). The values of the index in both groups were approximately the same before the beginning of treatment (p≥0.05) and indicated of pronounced inflammation in periodontal tissues. When patients were examined 7 days after the beginning of therapy, PMA index was 16.4±1.31% in patients of the treatment group and 14.2±1.21% in patients of the control group (p<0.05) (Table 3). Thus, there was a decreased inflammation in the periodontal tissues in both groups, but the PMA index values remained at a relatively high level. Upon the follow-up examination after 1 month, the PMA values in patients of the treatment group reached 12.2±1.27% and in the control group — 14.8±1.26% (p<0.05) (Table 3). During the patient examination 3 months after the conducted therapy, PMA index in the treatment group was 11.3±1.29%, while in patients of the control group — 15.4±1.42% (p<0.05).

When patients were examined 6 months after the treatment, the numerical values of PMA index were 9.7±1.02% (p<0.05) in patients of the treatment group and 14.6±1.2% (p<0.05) — in patients of the

***Table 1.*** *Dynamics of the Green Vermilion hygiene index (OHI-S) in patients of the treatment and control groups (M±m)*

| | Treatment Group | Control Group |
|---|---|---|
| Before therapy | 1,96±0,07 | 1,94±0,05 |
| 7 days | 0,39±0,03 | 0,4±0,03 |
| 1 month | 0,41±0,04 | 0,43±0,02 |
| 3 months | 0,61±0,02 | 0,59±0,03 |
| 6 months | 0,42±0,03 | 0,41±0,03 |
| 12 months | 0,64±0,03 | 0,67±0,03 |

***Table 2.*** *Dynamics of the bleeding index (PBI) in patients of the main and comparison groups (M±m)*

| | Treatment Group | Control Group |
|---|---|---|
| Before therapy | 3,30±0,09 | 3,26±0,03 |
| 7 days | 1,12±0,06 | 1,62±0,04 |
| 1 month | 0,76±0,04 | 1,22±0,06 |
| 3 months | 0,55±0,06 | 1,14±0,05 |
| 6 months | 0,50±0,04 | 1,17±0,06 |
| 12 months | 0,42±0,03 | 1,14±0,05 |

***Table 3.*** *Dynamics of the papillary–marginal–alveolar index (PMA) in patients of the treatment and control groups (M±m), %*

| | Treatment Group | Control Group |
|---|---|---|
| Before therapy | 3,30±0,09 | 31,4±2,1 |
| 7 days | 16,4 ± 1,31 | 14,2 ± 1,21 |
| 1 month | 12,2 ± 1,27 | 14,8 ± 1,26 |
| 3 months | 11,3 ± 1,29 | 15,4 ± 1,42 |
| 6 months | 9,7 ± 1,02 | 14,6 ± 1,2 |
| 12 months | 9,92 ± 1,42 | 13,28 ± 0,92 |

control group (Table 3), which indicates inflammatory progression and the need for repeated course of therapeutic and prophylactic measures.

During the dynamic examination of patients from both groups 12 months after treatment, it was found that the PMA values practically did not change ($p \geq 0.05$). Likewise, the values of PMA index were 9.92±1.42% and 13.28±0.92% ($p \geq 0.05$) in the treatment and control groups, respectively (Table 3). The obtained data demonstrate stability of results achieved by the dental gel application in treatment of patients with chronic simple catarrhal gingivitis and dental crowding.

The tendency towards decrease in the PMA index and its stabilization can be associated with the fact that regular appropriate personal oral hygiene together with courses of supportive therapy do not cause any cardinal disturbances in the periodontal tissues. At the same time, more pronounced and stable decrease in the PMA index values in patients of the treatment group ($p<0.05$) can be attributed to the greater efficiency of the dental gel based on original composition used in patients of this group as compared to the dental gel *Cholisal* applied in the control group.

## CONCLUSION

Thus, we conclude that the introduced dental gel exhibited significant clinical efficacy due to its anti-inflammatory, antimicrobial, regenerating and hemostatic effects, good fixation and uniform distribution on the gingiva surface and a convenient and hygienic application. None of the patients showed signs of intolerance, side effects or allergic reactions to the components of the dental gel.

The obtained results of the clinical efficacy of our dental gel provide the evidence to recommend it as a part of the complex therapy of chronic simple marginal gingivitis.

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# BIOMECHANICAL ASSESSMENT OF THE STRESS-STRAIN STATUS OF SPLINTING STRUCTURES AND TEETH PERIODONTIUM IN CASE OF CHRONIC PERIODONTITIS

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**ABSTRACT** — The authors have proposed an all-cast pin splint, whose technological feature is the ceramic lining of the over-the-bar part, which acts as a covering aesthetic structure, provides better distribution of the functional load and binds firmly mobile teeth affected by chronic moderate localized periodontitis.
The paper offers a view at the outcomes of a comparative analysis of the stress-strain state of periodontal tissues, teeth, and cortical bone in chronic moderate localized periodontitis at the anterior group of teeth in the lower jaw, when they are splinted with a specially designed splint and a conventional metal-ceramic monolithic splint by finite element modeling. The developed 3D mathematical model included, as the initial data, the features of the periodontium, of dental tissue and of cortical bone. There was an examination carried out focusing on the distribution of stresses, which occur when using the designed splint under the impact of multidirectional loads of 130 N, acting strictly down relative to the tooth longitudinal axis (vertically), and a load at an angle of 45°. The proposed method of splinting reduces the maximum stress in the periodontium at a vertical load by 26.9%, while at a side load of 45° it reduced the stress by 34.7%, if compared to a traditional monolithic metal-ceramic splint.

**KEYWORDS** — periodontium, periodontitis, splinting, metal-ceramic crown, stress-strain state.

## INTRODUCTION

There are numerous methods available for splinting mobile teeth in case of chronic localized periodontitis of varying degrees of severity [1–4], while there is no doubt that the top place among the methods employed to treat the pathology in question belongs to long-term splinting performed as a block of solid crowns or solid splints covered with ceramics [5]. The success of the orthopedic treatment will ultimately depend on the manufacturing technology and the splinting structure design.

Only the finite element method (FEM) is good for identifying the stress-strain state of objects featuring complex geometric shape [6]. Calculations based on FEM are in high demand, since they allow eliminating flaws at the design stage, reducing the time of refinement and the number of experiments (http://www.ansys.com/solutions/solutions-by industry/healthcare).

This work was performed using the ANSYS Academic Research Release 18.2 software package (academic license for scientific research; owner: S.P. Korolev Samara National Research University) which includes the ANSYS SpaceClaim geometric modeling module, and the ANSYS Mechanical module for solving strength problems. The consultation was offered by P.V. Bondarchuk, a CADFEM-CIS expert (Samara, Russia).

*Aim of the study* was to conduct a biomechanical evaluation of the efficacy of the proposed solid-cast ceramics-coated splint by the finite-element method on designed 3D mathematical model under multidirectional loads.

## MATERIALS AND METHODS

A CAD (automated drawing) model of the lower jaw was designed based on tomographic examination and its processing in the computer-aided design (NX) system. The model was modified through creating a crescent-shaped periodontal lesion zone at the front teeth area, while the lower jaw canines were modeled intact (Fig. 1a). The jaw model is divided into two volumes: the cortical and spongy bone. The model has holes for the teeth as shown in the step section (Fig. 1b).

The central incisor, the lateral incisor and the canine teeth were created following a 3D computer model. The study does not take into account the holes of missing teeth due to the lack of their effect on the model deformation. The periodontal layer is of the same thickness (0.25 mm [7]).

We have designed two types of splint models that connected the teeth of the original model. The study is

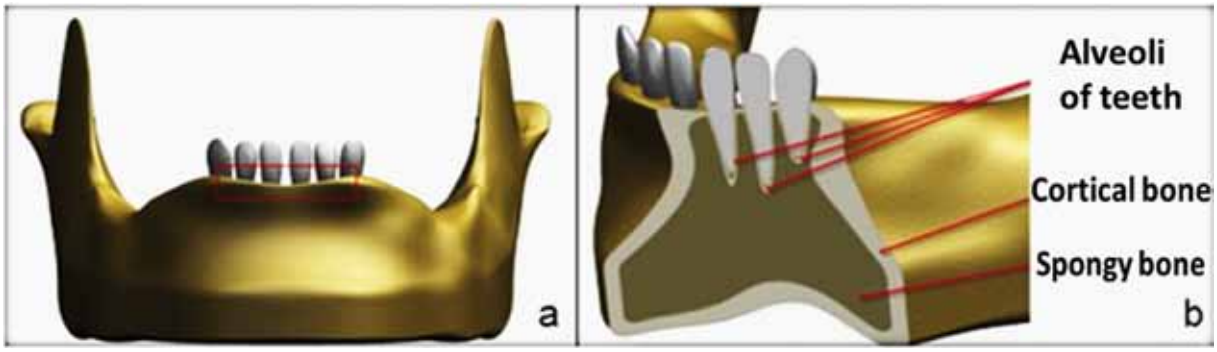


***Fig. 1.*** *Patient's lower jaw model: a — bone defect in the front teeth area; b — step section of the model*

focused on the conventional cermet monolithic splint (Group 1) and a splint of the new design (Group 2).

The newly designed splint (RF patent #175754) was installed on the dentition as shown in Fig. 2a. Fig. 2b offers a more detailed view of the design, showing the splint and the prepared teeth. The splinting technique implies manufacturing a metal frame, constructed as a cast beam with pins, whereas for the pulpless teeth with parallel canals, the length of the root pins was ⅔ the length of the root canals, and for teeth with non-parallel canals, or in case there was no way to unseal the canals properly, the pin length was up to ⅓ of the canal length, while they were parallel to each other and to all the canals of the pulpless teeth.

well as to the other pins, in order to fix the splint on the intact teeth. The visible part of the cast splint on the respective teeth is covered with a ceramics layer to match the color of the latter. The splint was fixed through cement (GC Fuji I) in the root canals and in the prepared grooves of the clinical dental crowns.

The model did not shape a ceramic layer due to a low impact on the structure rigidity determined by the metal frame.

Fig. 3a shows a cermet monolithic splint. Fig. 3b offers an image of a jaw with a partially shown prosthesis and the prepared teeth. The point of this method was as follows: a whole-cast prosthesis from metal-ceramic crowns was made for preprepared teeth, while

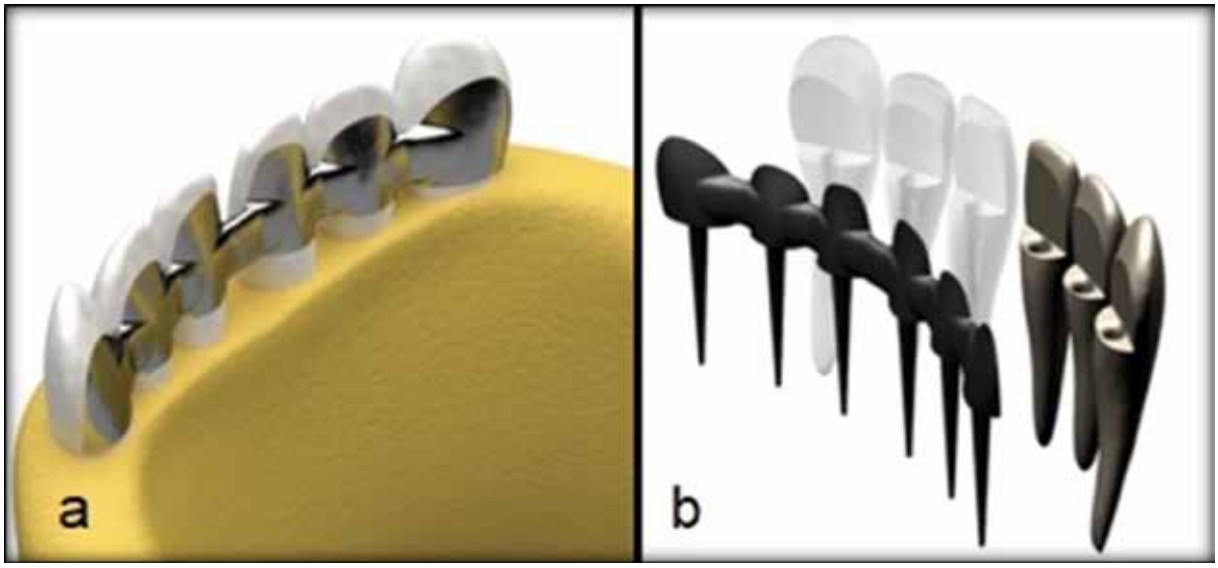


***Fig. 2.*** *Patented splint fitted to the lower jaw front teeth; a — splint, oral view; b — splint design*

The splint featured paired parapulpar pins up to 2–2.5 mm long, which were parallel to each other as

the prosthesis was manufactured from cobalt-chrome alloy to be further coated with ceramics through bak-

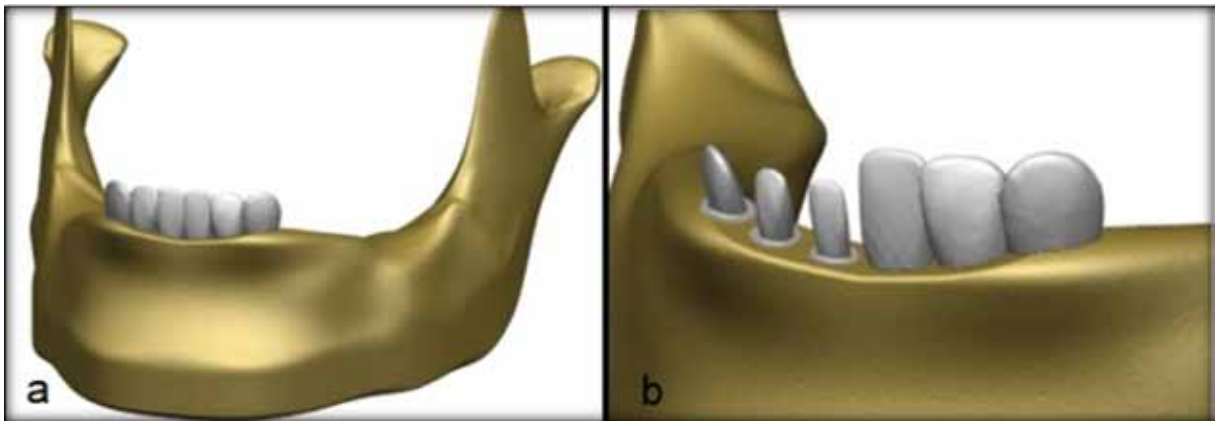


***Fig. 3.*** *Metal-ceramic monolithic splint: a — view of splint on the jaw; b — prepared teeth and the splint shown partially*

ing. The structure was fixed on the teeth stumps with cement (GC Fuji I).

Calculation of the stress-strain state relied on the data regarding the components of mathematical models (properties of the tooth, lower jaw cortical bone, periodontium) [8–12], as can be seen from Table 1.

***Table 1.*** *Properties of the materials employed through the study*

| Material | Young's module, MPa | Poisson's ratio | Strength limit | |
|---|---|---|---|---|
| | | | Compressive, MPa | Rupture, MPa |
| Cortical bone | 13700 | 0.26 | 156 | 85 |
| Periodontium | 0.6668 | 0.15 | 20 | 15 |
| Teeth | 19613.3 | 0.33 | 310 | 105 |

At the masticatory muscle (m. masseter) attachment point to the lower jaw, no vertical movement was allowed. When simulating biting, the models were loaded through applying force to the occlusal surfaces of the clinical dental crowns.

The strength analysis of the above models was performed with two loading options, hereinafter referred to as Step 1 and Step 2. The loading value was chosen so as to have a response at the masticatory muscle attachment area equal to 130 N. Given the model in question, this corresponded to a vertical load of 20 N per tooth. At Step 1 (Fig. 4a), a compressive load of 20 N was applied to the incisor surface of the teeth with a constant intensity, directed strictly downward relative to the tooth longitudinal axis (vertically), thus simulating food biting and chewing. At Step 2 (Fig. 4b), a load of 20 N was applied as well, featuring constant intensity at an angle of 45° relative to the longitudinal axis of the tooth [13].

The model was analyzed in relation to the stress distribution in the periodontium, dental tissues and cortical bone. Given that the analyzed materials feature different properties, several stress options were employed to assess the stress state. One was the equivalent of Von Mises stress for dental tissues, periodontium and the maximum major stresses (recommended for fragile bodies, such as cortical bone, for instance).

For a more detailed understanding, we obtained the results of stresses occurring in the periodontium, dental tissues, and cortical bone separately.

## RESEARCH OUTCOMES

***Results of the stress-strain state, Group 1.***

The maximum stresses in the periodontium under vertical load for Group 1 were: for the tooth 3.1 — 0.51 MPa; for the tooth 3.2 — 0.48 MPa; for the tooth 3.3 — 0.52 MPa (Fig. 5a). There is some uneven periodontium loading to be observed, while the maximum stresses at an angle of 45° were: for the tooth 3.1 — 0.92 MPa; for the tooth 3.2 — 0.95 MPa; for the tooth 3.3 — 0.86 MPa (Fig. 5b). The maximums localized on the periodontal dentoalveolar fibers (periodontium circular ligament).

The maximum stresses on the dental tissues in Group 1 under a vertical load for teeth 3.1 and 3.2 were 25.22 MPa; for tooth 3.3 — 22.17 MPa (Fig. 6a),

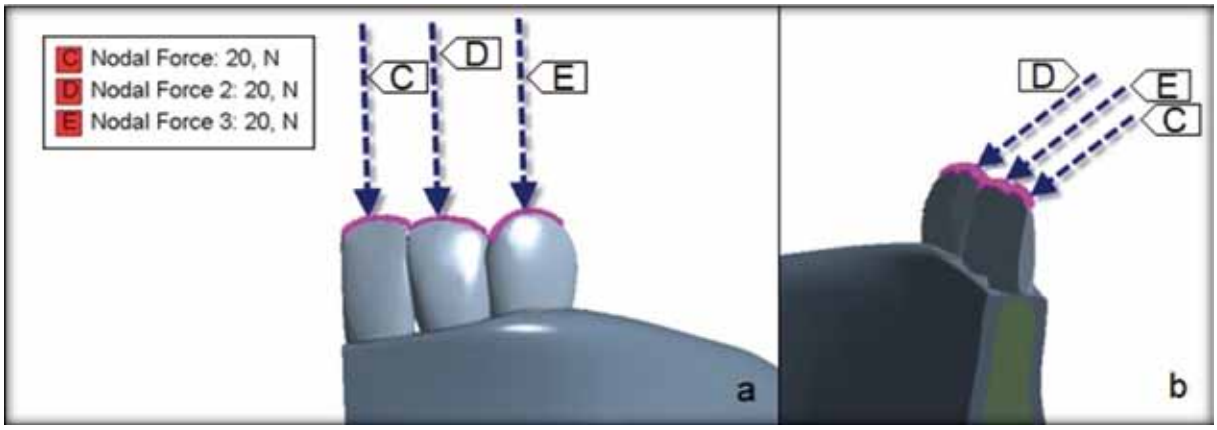


*Fig. 4. Areas of load application on the lower jaw teeth: a — step 1, vertical load; b — step 2, load at an angle of 45°*

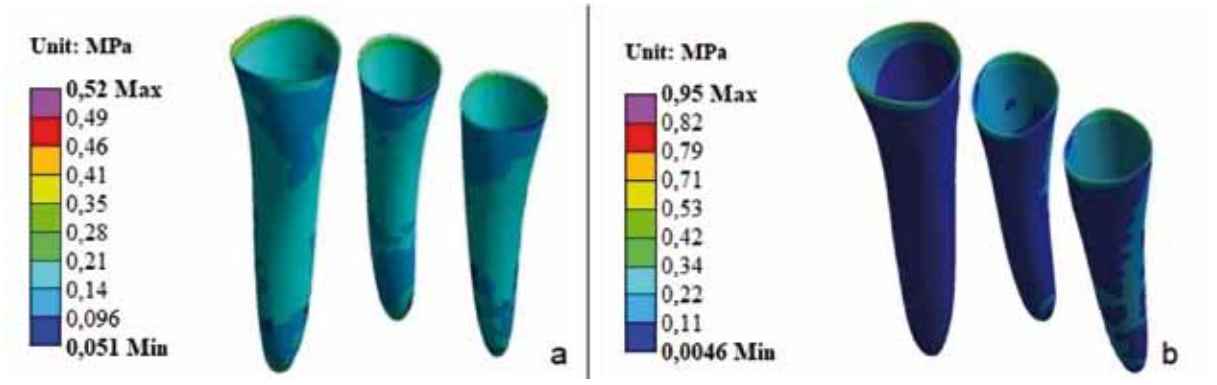


*Fig. 5. Maximum stresses occurring in the periodontium. Group 1: a — Step 1 (vertical load); b — Step 2 (45° load)*

while under a 45° load these were 59.81 MPa, 65.53 MPa and 68.35 MPa, respectively (Fig. 6b).

The maximum stress fields in the jaw cortical bone tissues in Group 1 were localized in the alveolar socket of tooth 3.3 and were 10.92 MPa; for tooth 3.2 — 9.15 MPa, and for tooth 3.1 — 10.11 (Fig. 7a). The maximum stresses at an angle of 45° in the cortical bone were 21.35 MPa for the teeth 3.3, 3.2 — 21.12 MPa; for 3.1 — 19.73 MPa (Fig. 7b).

***Results of the stress-strain state, Group 2.***

Fig. 8a shows the results of the maximum major stresses in the periodontium in Group 2: for the tooth 3.1 — 0.35 MPa; for the tooth 3.2 — 0.35 MPa; for the tooth 3.3 — 0.38 MPa. Under a loading at an angle of 45°, there was already a uniform loading to be observed in the periodontium, while the maximum stresses were: for the tooth 3.1 — 0.61 MPa; for the tooth 3.2 — 0.62 MPa; for the tooth 3.3 — 0.62 MPa (Fig. 8b).

The maximum stresses on the dental tissues in Group 2 under a vertical load for the tooth 3.1 were 18.55 MPa; for the tooth 3.2 — 18.53 MPa, and for the tooth 3.3 — 17.65 MPa (Fig. 9a), at an angle of 45°, the load on the tooth 3.3 was 44.25 MPa; on the tooth 3.2 — 44.23 MPa, while on the tooth 3.1 it was 44.23 MPa (Fig. 9b).

The maximum stress on the lower jaw model cortical bone in Group 2 under a vertical load for tooth 3.3 was 7.35 MPa; for tooth 3.2 — 7.31 MPa, and for tooth 3.1 — 7.28 MPa (Fig. 10a). The maximum stress at 45° in the cortical bone was 13.97 MPa for the tooth 3.3; for the tooth 3.2 — 13.16 MPa, and for the tooth 3.1 — 13.73 MPa (Fig. 10b).

Tables 2–4 offer a comparative analysis of the obtained quantitative indicators for the maximum stresses depending on the design and the part of the system.

The data in Table 2 shows that the resulting maximum stresses in the periodontium are high in Group 1, where the monolithic metal-ceramic splint was used. These indicators were the highest for both the vertical load (0.52 MPa) and the lateral (45°) load

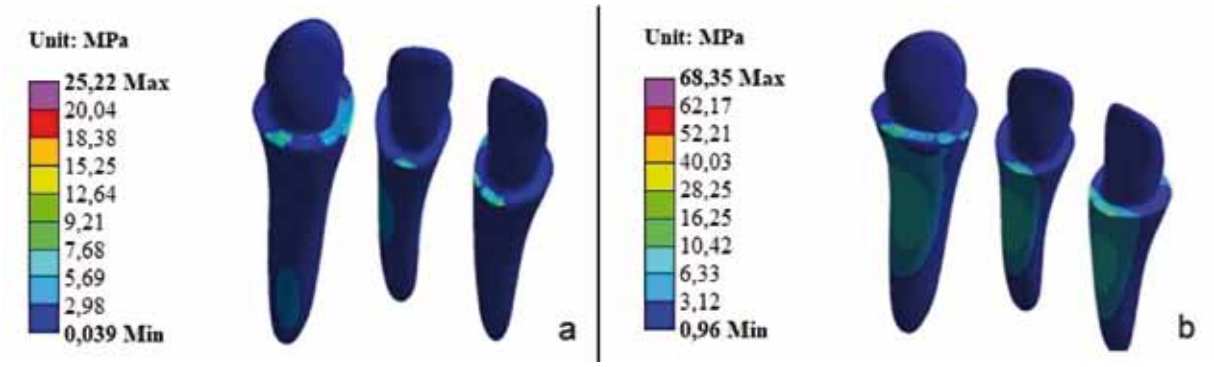


*Fig. 6. Maximum stresses occurring on the dental tissues. Group 1: a — Step 1 (vertical load); b — Step 2 (45° load)*

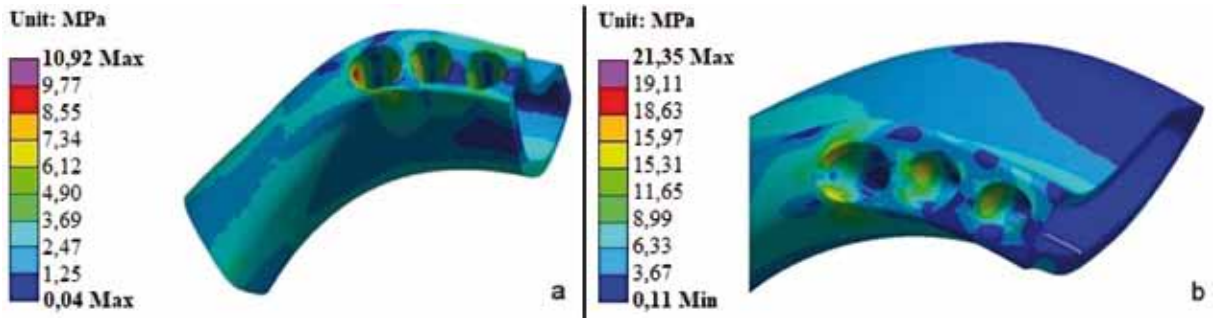


*Fig. 7. Maximum stress on the jaw cortical bone. Group 1: a — Step 1 (vertical load); b — Step 2 (45° load)*

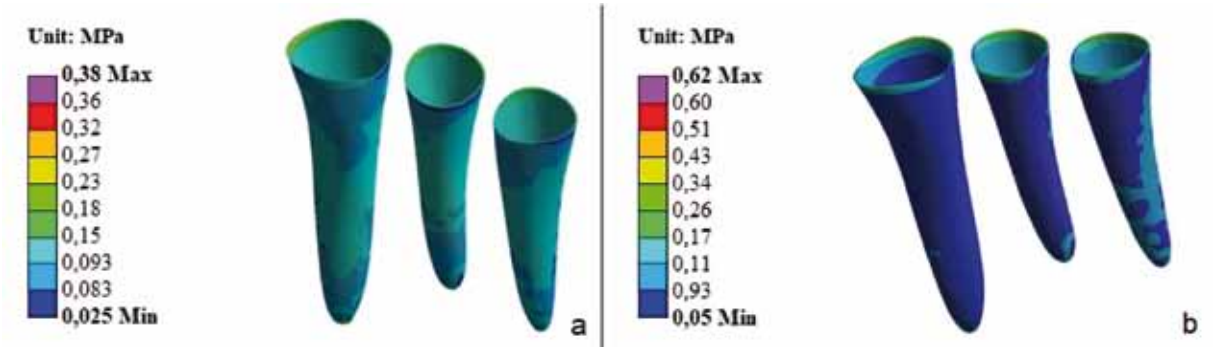


*Fig. 8. Maximum stresses occurring in the periodontium. Group 2: a — Step 1 (vertical load); b — Step 2 (45° load)*

(0.95 MPa). The use of the proposed splint allowed reducing the occurrence of maximum stresses in the periodontium, as well as distributing the equal load delivered through the structure to the periodontium. Mathematical analysis in Group 2, therefore, revealed a 26.9% decrease in this value under the vertical load (0.14 MPa), while under the lateral load the decrease was 34.7% (0.33 MPa), if compared to Group 1.

The obtained values of maximum stresses in dental tissues (Table 3) under the vertical load (90°), were the highest in Group 1 (25.22 MPa) and the lowest in Group 2 (18.55 MPa), with the statistically significant difference between the groups 26.4% (6.67 MPa). In case of the lateral load of 45°, the maximum stresses in the tooth tissues in Group 1 were 68.35 MPa, and in Group 2 — 44.25 MPa. Given the fact that the critical stresses in Group 2 proved higher for both loading options, the destruction of tooth tissues would occur faster while using a monolithic metal-ceramic splint.

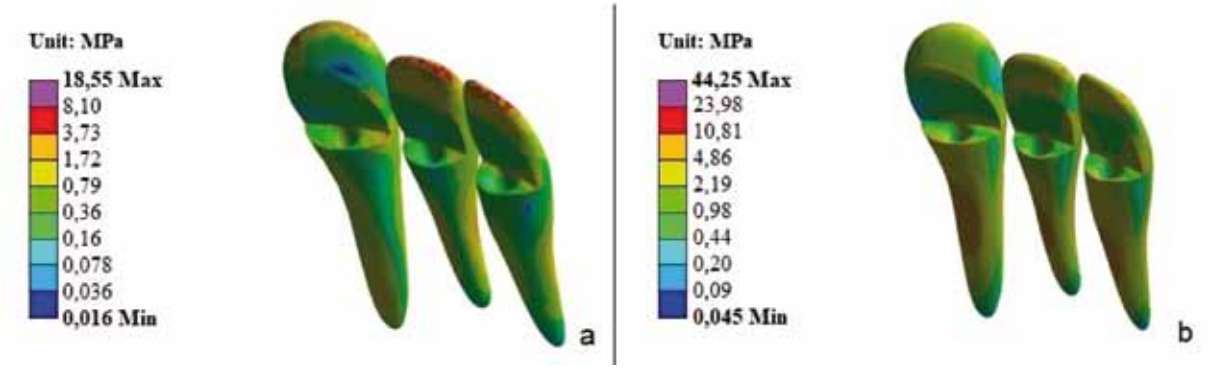


*Fig. 9. Maximum stresses occurring on the dental tissues. Group 2: a — Step 1 (vertical load); b — Step 2 (45° load)*

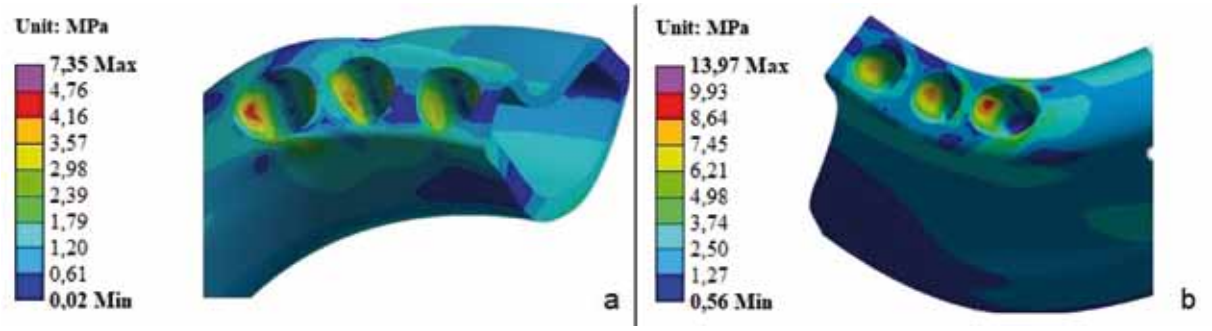


*Fig. 10. Maximum stresses occurring on the cortical bone. Group 2: a — Step 1 (vertical load); b — Step 2 (45° load)*

***Table 2.** Results of maximum stresses in periodontium*

| Group | Maximum stress, MPa | | | | | | Critical stress resulting in periodontium trauma, MPa |
|---|---|---|---|---|---|---|---|
| | 90° | | | 45° | | | |
| | 3.1 | 3.2 | 3.3 | 3.1 | 3.2 | 3.3 | |
| 1 | 0.51 | 0.48 | 0.52 | 0.92 | 0.95 | 0.86 | 18 |
| 2 | 0.35 | 0.35 | 0.38 | 0.61 | 0.62 | 0.62 | |

***Table 3.** Results of maximum stresses in dental tissues*

| Group | Maximum stress, MPa | | | | | | Critical stress resulting in tooth collapse, MPa |
|---|---|---|---|---|---|---|---|
| | 90° | | | 45° | | | |
| | 3.1 | 3.2 | 3.3 | 3.1 | 3.2 | 3.3 | |
| 1 | 25.22 | 25.22 | 22.17 | 59.81 | 65.53 | 68.35 | 22 |
| 2 | 18.55 | 18.53 | 17.65 | 44.23 | 44.23 | 44.25 | |

The results of maximum stresses occurring in the cortical bone (Table 4) under the vertical load and at an angle of 45° were highest in Group 1 — 10.92 and 21.35 MPa, respectively. In group 2, the vertical and the lateral loads entailed maximum stresses of 7.35 and 13.97 MPa, respectively, which is 32.6 and 34.5% below similar values in Group 1, which indicates rather a significant load in the cortical bone, and in case of bending loads there is destruction possible of the cortical bone marginal zones in the Group 2.

***Table 4.** Results of maximum stresses in cortical bone*

| Group | Maximum stress, MPa | | | | | | Critical stress resulting in cortical bone trauma, MPa |
|---|---|---|---|---|---|---|---|
| | 90° | | | 45° | | | |
| | 3.1 | 3.2 | 3.3 | 3.1 | 3.2 | 3.3 | |
| 1 | 10.11 | 9.15 | 10.92 | 19.73 | 21.12 | 21.35 | 73 |
| 2 | 7.35 | 7.31 | 7.35 | 13.73 | 13.16 | 13.97 | |

## CONCLUSION

In view of our outcomes of the stress-strain state analysis performed using 3D mathematical models of lower jaws with chronic moderate localized periodontitis, splinted with constructions of various design, the following conclusions can be made:

1. The stress distribution pattern of all the types of splinting structures examined above corresponds to the nature of the stress distribution typical of rigid bodies with a predominant load concentration on the tooth circular ligament. Significant stress reduction in the patented design, if compared to the conventional splint, is due to significantly reduced stresses at the *tooth–splint* boundary subjected to a vertical and a lateral load.

2. Using the patented splint allows a significant reduction in the equivalent stresses not only on the border of different tissues, in the dentine, in the tooth root, yet also in periodontal tissues as well as in the cortical bone with no risk of dangerous load concentration, thus minimizing the likelihood of overload that the teeth may be subjected to.

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# DEVIATIONS IN THE POSITION OF THE THIRD MOLARS

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**ABSTRACT** — Timely teething is one of the most important indicators of the harmonious development of the dentoalveolar system, which is determined by the correct (physiological) position of the teeth in the dentition, ensuring the optimal shape and function of the temporomandibular joint, the work of the chewing muscles, the height of the lower part of the face, and aesthetics of a smile. Retention of individual teeth is often the reason for the formation of anomalies in the dentition, their closure, functional and aesthetic disorders. An urgent problem in modern dentistry is the problem associated with the development of third molars. According to the results of the analysis of 3000 orthopantomograms of the jaws of patients 7–25 years old with dentoalveolar anomalies, the spatial arrangement of the primordial of the third molars relative to the buttresses of the upper and lower jaws was studied. It was found that the buttresses on the upper jaw are vertical and do not interfere with the correct eruption of the third molars. The buttresses located near the primordial of the third molars on the lower jaw contribute to the retention of the third molars due to the change in inclination during the formation stage.

**KEYWORDS** — third molar, eruption pathology, dystopia, retention, wisdom tooth, lower jaw, retromolar space.

## INTRODUCTION

Currently, the prevalence of retention of lower third molars ranges from 35 to 50% [4, 20, 23].

Anomalies in the development and eruption of these teeth lead to the formation of bone pockets, destruction of hard tissues of the adjacent tooth, the formation of follicular cysts, the development of neuralgic pain, osteomyelitis, phlegmon, sepsis [1, 7, 12, 15, 18, 22, 25, 32, 38, 40].

Complications of obstructed eruption of the lower wisdom tooth proceed according to the type of inflammatory reactions and depend on the anatomical and physiological characteristics of the retromolar region [3, 16, 28, 33, 39, 41]. The increase in the prevalence of retention of third molars is due to the general reduction of the dentition in the process of phylogenesis [6, 31, 35].

For many years, question of the influence of third molars on the occurrence of maxillofacial anomalies has been intensively studied by both Russian and foreign specialists [5, 8–10, 14, 21, 26, 29, 30, 36].

According to the literature, the formation of the upper and lower jaws, various groups of teeth is in direct proportion to the growth and development of wisdom teeth. This indicates that the eruption of third molars has a direct effect on the structure of the entire dentoalveolar system [2, 11, 17, 19, 24, 27, 34, 37].

There is no consensus about the influence of abnormally located third molars on the occurrence of maxillofacial anomalies or their recurrence after the orthodontic treatment. According to S.P. Atkinson (1950), the excessive pressure exerted by third molars on the anterior teeth leads to excessive eruption of the second molars. In some cases, it can cause an *open bite* and traumatic occlusion of the second molars. S. Mueg (1992) assigns a decisive role to the influence of growth of these teeth on the development of crowding of teeth in the anterior segment, while Schwarz (1975) and Ades (1990), on the contrary, and do not consider this factor to be significant.

Problems with third molars attract the attention of dentists in many countries around the world. In 1998, a national congress in Great Britain summarized the experience of working on this issue in the largest clinics in the country for 10 years. Particular attention was paid to partially or completely impacted third molars. The issue of their removal is recognized as not a preventive measure, but considered as a method of treatment. In 1999, at the National Institutes of Health conference in the USA, the role of third molars in the growth and development of the dentition, indications for extraction and the most appropriate age for removing the germs of these teeth was discussed. It is recognized that in order to prevent the development of crowding of teeth, the removal of the germs of the third molars should be considered very appropriate, and the most suitable age for this is over 10 years, i.e.

until the period of complete formation of the roots of these teeth.

N.P. Stadnitskaya (2009) found that the beginning of the formation of the germs of third molars should be considered the age from 6 to 16 years. In 22.4% of cases, the germs of third molars in the same patient may begin to form at different times. Differences in the stage of formation are noted in the first half of the formation of the coronal part of the rudiment. By the end of the formation of roots, the differences are leveled. Also, author noted that anomalies of the germs of third molars (position, shape and size, macroscopic structure) occur in 18.7% of cases, and just as anomalies of eruption are complicated by inflammatory diseases (0.3%), destruction of the surrounding bone tissue and hard tissues of the second molars (0.9%), partial or complete impaction of the second molars (1.2%), the development of crowding of the teeth in the frontal parts of the jaws (17%) or its recurrence at the end of orthodontic treatment (12%). At the same time N.P. Stadnitskaya (2009), on the basis of an X-ray examination, identified four main types of position of the impacted third molars: medial (47%), horizontal (29%), vertical (21%), distal (3%), which cause complications characteristic of each type of position

Salakh S.M. Temeza, A.P. Romanovskaya (2010) in the course of a study found that a lack of space for the eruption of third molars over 3 mm and angulation of more than 69 degrees are an indication for their removal. The optimal period for the removal of third molars is 13–15 years old. After removal of the third molars, a significant decrease in the terms of orthodontic treatment and stable results in the retention period are observed.

S.S. Almurat, B.B. Aimukhanbetov (2015) conducted a study and found that the frequency of tooth impaction in childhood was 27%, the most common impaction of third molars was 44%, of which in a horizontal position - 48%. In the majority of clinical observations, bilateral impaction of the third mandibular molar was observed — 18.7%.

I.N. Skapkareva, O.A. Zhigalsky (2014) did not reveal the influence of gender on the eruption of the third molar. Shift in the timing of the eruption of the third molars to later ones was noted. In a modern person, a gradual rudimentation of the third molar occurs, in 76.7% of people, the eighth teeth do not erupt.

I.A. Ganiev (1993), S.B. Fishchev et al. (2008), G.A. Bonetti (2008) discussed the effect of third permanent molars on crowding and recurrence after orthodontic treatment. E.A. Bragin (2006) noted that one of the informative methods for diagnosing anomalies is orthopantomography of the jaws and A.P. Kibkalo (2008) proposed methods for analyzing orthopantomograms based on standard morphometric symmetrically located points.

S.B. Fishchev et al. (2012) proposed a technique for examination of orthopantomograms, which makes it possible to determine the position of the third permanent molars: on the image, according to the criterion of information content, lines are drawn along the coordinate points. The main horizontal plane was a line connecting the lower edges of the slopes of the articular tubercles (T). From the middle of the T-T line, a perpendicular was lowered and a median vertical line was drawn, which was designated as the line of the aesthetic center, which passed between the medial incisors of the upper and lower jaw and through the *Me* point on the chin. Tangent lines were drawn on both sides along the lower edge of the lower jaw body and along the outer edge of the lower jaw branch. The point of intersection of the bisector of the angle formed by the tangent lines to the angle and body of the mandible with the inner angle of the mandible is designated as the retromolar point (RM), and the bisector itself was regarded as the *stress axis*. The position of the third molar beyond the retromolar point or *stress axis* was regarded as critical for the normal eruption of wisdom teeth.

N.V. Pankratova et al. (2014) studying the frequency of spreading and position of third molars at the stages of their formation in 866 OPTG of patients 7–18 years old. The presence or absence of germs of 3 molars was recorded in 4 age groups: 7–9 years, 10–12 years, 13–15 years and 16–18 years. On the panoramic image, the position of the 3 molars was assessed by the size of the internal angles formed by the perpendicular from the line connecting the apexes of the distal and mesial cusps of the 3 molars: for the upper — to the infraorbital line, for the lower ones — to the plane of the base of the lower jaw. The largest number of panoramic images with 3 molars was found in patients aged 10—15 years. The magnitude of the angles characterizing the position of the 3 upper molars decreases with age, and the lower ones increase.

During comparative characteristics of the position of the third molars in various occlusion anomalies in patients from 7 to 25 years old with a narrowing of the dentition N.V. Pankratova et al., (2016) found that the angles of inclination of the third molars change their magnitude: on the upper jaw, the indicators decrease, on the lower jaw, they increase with varying degrees of reliability. Third molars tend to take a vertical position even with anomalies of size of the dentition, and the stages of formation of third molars increase with age with a high coefficient of reliability between the corresponding indicators in age groups.

The magnitude of the angles characterizing the position of the third molars in patients 15–18 and 18–25 years old significantly differs from their values in the initial state, in patients aged 7–12 years. At the age of 15 to 25, teeth are actively formed and, as a result, the third molars change their position. Active changes in the position of the teeth occur before the age of 18, and from 18 to 25 years, the position of the third molars changes, but the magnitude of the angles confirming these changes is unreliable.

However, in all of the literature sources we studied, issues of impaction of third molars and methods of studying their position are discussed. Recommendations are given on the methods and timing of removal of third molars. At the same time, there is no indication of the reason for the incorrect position of the third molars during their formation and eruption.

The purpose of this study is to try to find the reason for the change in the position of third molars during their formation and eruption.

## MATERIALS AND METHODS

During the study of this problem, we analyzed more than 3000 orthopantomograms of the jaws of patients from 7 to 25 years old with various anomalies of the maxillofacial system (crowded position of the teeth, malocclusions class II and III, transversal occlusion). The position of the molars was assessed by the size of the internal angles (1, 2, 3 and 4) formed by the line connecting the tops of the cusps of the third molars and perpendicular to the infraorbital line for the teeth of the upper jaw and to the plane of the base of the lower jaw for the teeth of the lower jaw (Fig. 1).

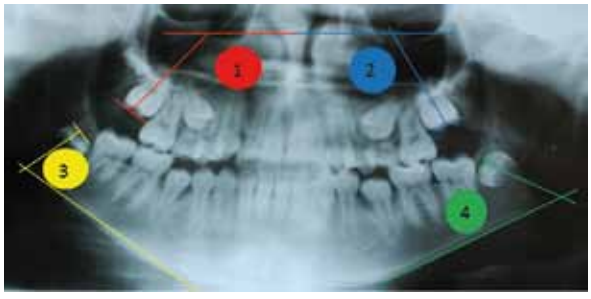


***Fig. 1.*** *Determination of the angles characterizing the position of the third molars of the upper (1 and 2) and lower (3 and 4) jaws*

Were selected 105 orthopantomograms of the jaws of patients for a long time under dispensary observation up to 12 years, who had from 2 to 8 X-ray images. Studying the dynamics of the formation of third molars in the same patient, we drew attention to the severity of the buttresses of the upper and lower jaws.

There are 4 buttresses on the upper jaw: frontal-nasal; alveolar-zygomatic; pterygoid palatine; palatine. ***The frontal-nasal buttress*** rests at the bottom on the alveolar eminences in the canine region, at the top it continues in the form of a reinforced plate of the frontal process of the upper jaw, reaching the nasal part of the frontal bone. Balances the upward pressure exerted by the canines. ***The alveolar-zygomatic buttress*** goes from the alveolar eminence of the 1st and 2nd molars, goes up the zygomatic ridge to the zygomatic bone, which redistributes the pressure. ***The pterygo-palatine buttress*** starts from the alveolar eminence of the molars and the tubercle of the upper jaw, goes up, where it is reinforced by the pterygoid process of the sphenoid bone and the perpendicular plate of the palatine bone. Balances the force exerted by molars from bottom to top and back to front. ***The palatine buttress*** is formed by the palatine processes of the upper jaw and the horizontal plates of the palatine bone, connecting the right and left alveolar arches in the transverse direction. There are 2 main buttresses on the lower jaw: 1 — ***alveolar***; 2 — ***ascending*** (along the branch of the lower jaw) (Fig. 2).

Thus, the buttresses of the upper jaw, in particular the alveolar-zygomatic buttress, goes from the alveolar eminence of the 1st and 2nd molars, goes up the zygomatic ridge to the zygomatic bone, which redistributes the pressure and the pterygo-palatine buttress starts from the alveolar and tubercle of the molar of the upper jaw, goes up, where it is reinforced by the pterygoid process of the sphenoid bone and the perpendicular plate of the palatine bone. As indicated by T.S. Guseinov, M.A. Azizov (2016) the biomechanism of the temporomandibular joint and the thin histotopographic structure of the lower jaw make it possible to identify 7 thickenings of the lower jaw or directions of the bone beams (buttresses). The following thickenings are distinguished:1 — from the recess behind the last molar to the corner of the lower jaw; 2 — from the corner of the lower jaw along the posterior edge of the branch of the jaw to the temporal process; 3 — from the place of attachment of the masticatory muscles to the processes: temporal and alveolar and articular; 4 — between the chin tubercles; 5 — from the base of the lower jaw to the temporal and articular processes; 6 — along the posterior edge of the lower jaw branch; 7 — between the temporal and articular processes [13].

The formation and development of buttresses begins at 4–5 years and reaches its development in adulthood (20–65 years) and their thinning begins at 65–70 years in connection with age-related and senile atrophy of the jaw bone plates.

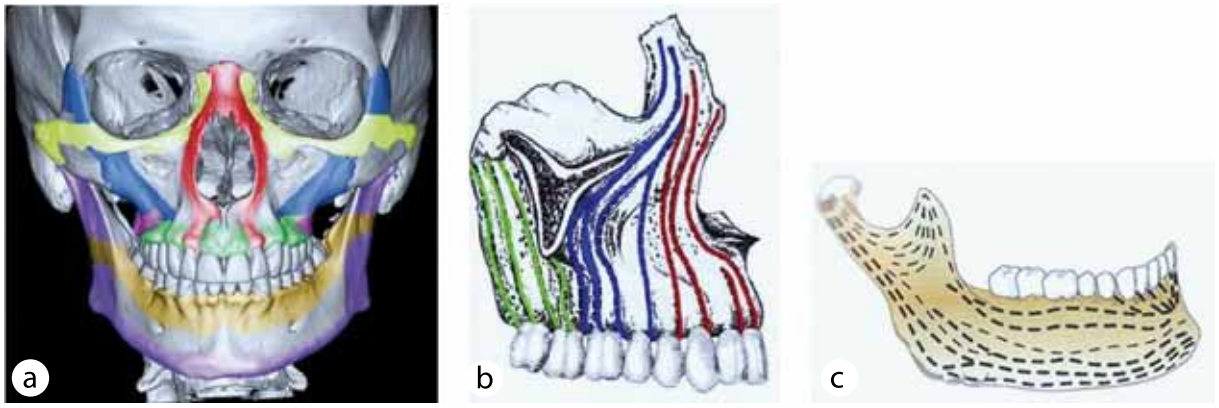


***Fig. 2.*** *a — buttresses of the skull, b — buttresses of the upper jaw, c — buttresses of the lower jaw*

## RESULTS AND DISCUSSION

As already mentioned, we divided the orthopantomograms of the jaws into groups according to the location of the roots of the third molars of the lower jaw: with a distance from the buttresses (Fig. 3) and without a distance (Fig. 4). It is noted that the third molars, located with a distance from the buttresses, increase the angle characterizing the position of the teeth with age (Table 1).

As follows from Table 1, the presented values of the angles characterizing the position of the third lower molars, depending on the location of their roots relative to the buttresses, indicate a decrease in the angle of inclination of the molars in the case of a close passage of the buttresses and an increase in the presence of a distance between them.

In confirmation of the above noted feature, the graphs in Fig. 5 and 6 are presented.

In the course of visual examination of the orthopantomograms of the jaws, a difference in the position of the third molars of the lower jaw was noted (Fig. 7).

The results of X-ray studies formed the basis for an in-depth study of the morphology of the skull buttresses, which was described earlier. The data obtained logically explains the minimum number of problems with the position and eruption of the third molars of the upper jaw. The buttresses of the upper jaw are positioned vertically, creating favorable conditions for the formation of the correct direction and position of the third molars during eruption. This cannot be asserted when evaluating information about the lower molars, including a significant amount of their retention. The close location of the roots of the third molars of the lower jaw to the buttresses worsens their position with age (changes in the angles of inclination), prevents their eruption, contributing to retention.

## CONCLUSION

1. The results of clinical and radiation studies based on the anatomical and topographic features of the upper and lower jaw, allow us predicting the possibility of complications during the eruption of third molars.

2. During the study, the location of the buttresses of the skull including the upper and lower jaw was studied.

3. It has been established that the location of the primordial of third molars related to the buttresses is important for their position and eruption.

4. The buttresses on the upper jaw are vertical and do not interfere with proper eruption.

5. Buttresses on the lower jaw can pass near the primordial of third molars, depending on the distance between them, their retention is possible due to a change in their inclination during formation.

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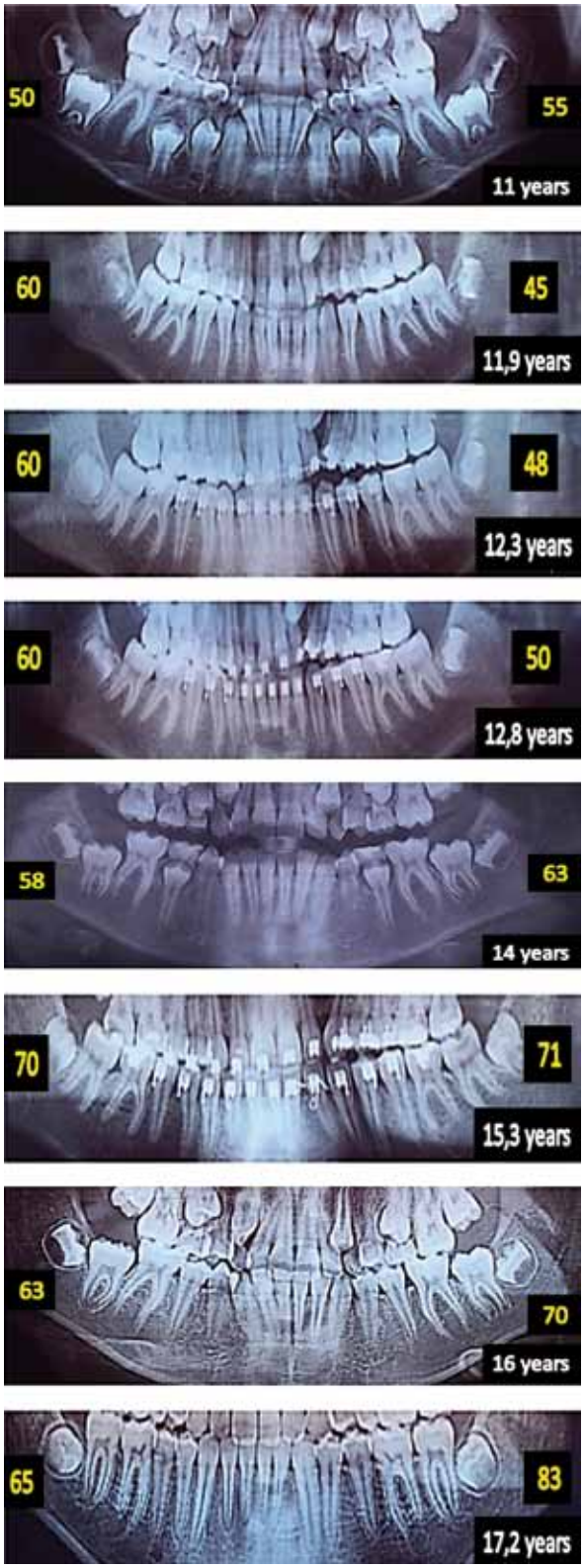


*Fig. 3. A selection of orthopantomograms of the jaws, with the location of the roots of the third molars with a distance from the buttresses (the angles of the 3 molars are shown in yellow, the age of the patient is indicated in white)*

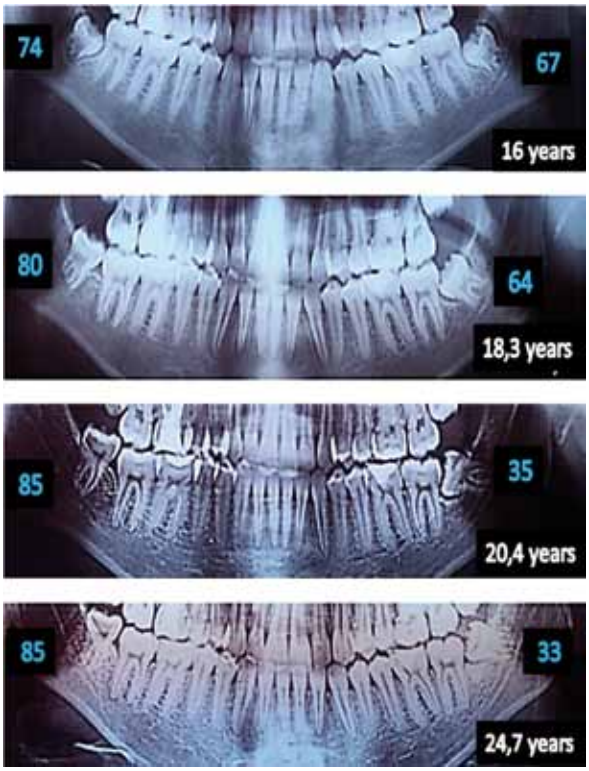


*Fig. 4. A selection of orthopantomograms of the jaws, with the location of the roots of the third molars without distance from the buttresses (the angles of the 3 molars are indicated in blue, the age of the patient is indicated in white)*

***Table 1.*** *Average values of the angles of inclination of the third molars of the lower jaw, with different positions of the roots of the teeth from the buttresses, (°)*

| Age of the patient, (years) | The angle of inclination of the lower third molars, (°) | |
|---|---|---|
| | With distance | Without distance |
| 12 | 52,5 | 60,5 |
| 13 | 54,5 | 75,5 |
| 14 | 57,5 | 65,5 |
| 15 | 60,5 | 68,1 |
| 16 | 67,5 | 70,7 |
| 17 | 67,7 | 63,3 |
| 18 | 73,0 | 56,0 |
| 19 | 74,0 | 44,6 |
| 20 | 75,1 | 44,3 |
| 21 | 83,1 | 41,7 |
| 22 | 83,7 | 39,0 |
| 23 | 84,0 | 40,1 |
| 24 | 83,1 | 39,5 |
| 25 | 84,0 | 39,8 |

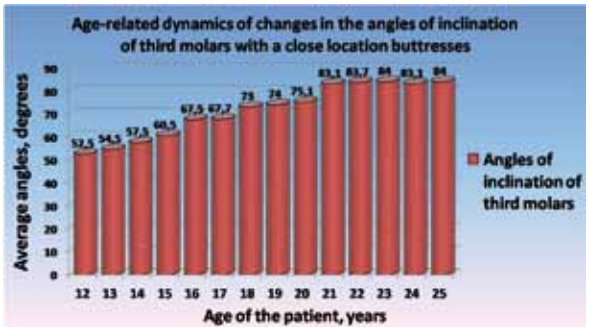


*Fig. 5. Graphical representation of age-related dynamics of changes in the angles of inclination of the third molars of the lower jaw with a close location to the buttresses*

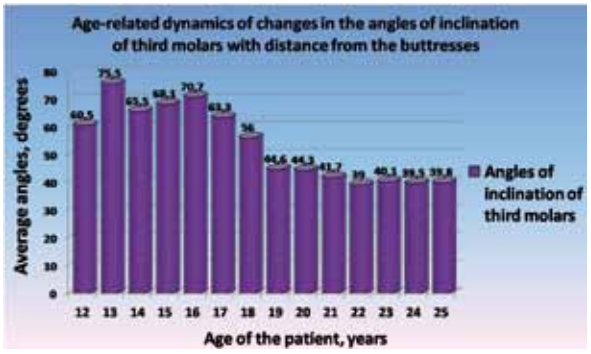


*Fig. 6. Graphic representation of age-related dynamics of changes in the angles of inclination of the third molars of the lower jaw with a distance from the buttresses*

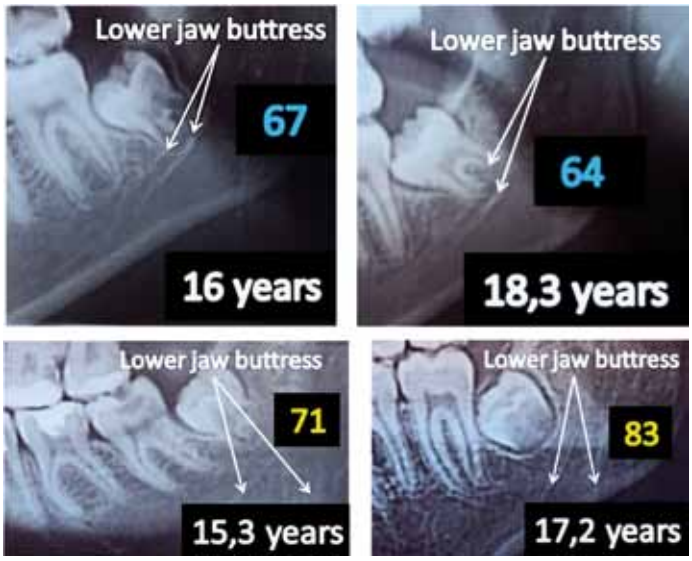


*Fig. 7. A selection of orthopantomograms of the jaws with the location of the roots of the third molars and the values of their angles without distance (a, b) and with distance (c, d) from the buttresses (white color — patient's age)*


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# CREATING OSTEOPLASTIC MATERIALS TO REPAIR JAW BONES DEFECTS

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**ABSTRACT** — In the course of our research we have developed a technology for fabricating an osteoplastic material from natural bone tissue. The obtained preparation in the form of gel contains hydroxyapatite, calcium triphosphate compounds and stimulators for regeneration. The proposed experimental material produces a stimulating effect on the growth of animal cell types; it enables to produce bioactive materials with increased biocompatibility. Application of the experimental gel facilitates the process of bone-tissue regeneration in the laboratory animals, which, in its turn, confirms the optimal composition of the material. We have established that during the integration of our osteoinductive material the defect zone is completely replaced by the bone tissue.

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

## INTRODUCTION

Today, osteoplastic materials are widely used to repair bone defects in maxillofacial surgery and dental surgery. Due to the bone resorption and alveolar bone atrophy the alveolar crest height decreases by 7 mm after one year with about 50% left from the original volume. That is why there is an acute need for replanting of an osteoplastic material aiming at performing a dental implant procedure. After the operation it is necessary to repair the dental bone defect of the jaw. The main problem of implant treatment is not only installation of a right-sized implant, but the build-up of the necessary volume of hard and soft tissues around it, what leads to an esthetic and functional result [1].

For this purpose, auto-, allo- and xenografts are used.

Currently available evidence has shown that the autogenous bone has the highest osteoinductive potential, but there are some disadvantages: a risk of resorption, a complicated preservation procedure, a necessity of inflicting an additional injury to a patient during sampling as well as a biological incompatibility of donor and recipient.

The use of allografts or xenograft entails the risk of immune conflict, which may result in rejection and attachment of secondary infection. [1, 2].

To date a limited line of bone replacement materials is brought to market which are applied in maxillofacial surgery, dental surgery and traumatology in general. Bio-Oss (Switzerland) and Osteo-Biol (Italy) are the leaders among the imported materials. These products have 98% of survival rate and higher, but they are several times more expensive than domestic analogues. Local osteoinductive materials are relatively inexpensive but they have significant disadvantages:

— lack of flexibility;
— low level of biocompatibility [3].

Therefore, it is highly desirable to produce an osteoplastic material which would be a mix of bioavailable components of bone matrix in combination with sources of energy and stimulation of osteocyte division and proliferation.

*Aim of the work* is to create and investigate a new internationally competitive material, fully bioinert and biocompatible with the body tissues, capable of replacing and compensating for jaw bones defects. For the first time, its composure will contain nanoparticles of silicon dioxide and minerals.

## MATERIALS AND METHODS

Bone powder obtained by pretreatment of the tubular bones of slaughter bulls was used as components. The femoral diaphysis was stripped of muscles and ligaments and the cortical layers were sawed off, exposing the spongy bone. Spongy bone tissue was crushed in a hammer mill (Molot 200/800) to a particle size of 1–1,5 $cm^3$. Then the bone blocks were placed in a 2% solution of sodium chloride for 24 hours, washed with water, then placed for hydrolysis in a 0,4 n solution of sodium hydroxide for 24 hours; the blocks were washed with distilled water until complete neutralization of sodium hydroxide. After washing the blocks, they were degreased in chloroform and acetone for 48 hours, after which the blocks were washed again with purified water while constantly stirring on a magnetic stirrer. An intermediate control was performed: the blocks were stained with Sudan for the presence of fat and lipoproteins. Until a negative reaction result is obtained. Then the composition

was calcined in a muffle furnace (ELF 11/6), evenly increasing the temperature from 100 to 300° C for 5 hours. An intermediate control was performed — for Lowry-Barnstead protein. The negative reaction result served as a criterion for moving to the next technological process. Then an intermediate quality control was performed on the mineral component. It is carried out with a standard 1% $H_2SO_4$ solution. The obtained blocks were dissolved without residue and without sediment. The blocks of the bone mineral component obtained in this way are cream-colored blocks consisting of a compound of natural hydroxyapatite with phosphates and carbonates. The resulting blocks were crushed on an ultrasonic homogenizer (MEF93.1) to a powdery state and sterilized. As the most effective methods of sterilization of the obtained bone powder, the air method of sterilization (180° C — 30 minutes) in a drying cabinet ШС 35/250-250-П-Standard and microwave radiation in a microwave sterilizer LS-B701 were chosen. Sterilization control was performed using physical and chemical methods. The physical method of monitoring the operation of sterilizers was carried out by using control and measuring devices that record temperature, pressure and time. The chemical method consisted in the use of chemical tests and thermochemical indicators, which were placed in the sterilization chamber at control points for each tab of materials, both outside the packages and inside the packages. The effectiveness of sterilization was controlled by a bacteriological method, using biotests of sterilization, which are objects seeded with test microorganisms. The bacteria Bacillus licheniformis strain G VKM B-1711D in the amount of $n \cdot 10^6$ are used as test microorganisms. They are highly resistant to heat, so they are used to control sterilization. Based on the death of test microorganisms, a conclusion was made about the effectiveness of the process.

After sterilization, the powder was mixed with glucosamine and a premix containing minerals and vitamins (observing the rules of asepsis). To obtain a paste-like mass, a hyaluronic acid gel was added to the mixture with constant stirring, and mixed until a uniform cream-colored viscous-plastic mass was formed. For 3 months, 60 Wistar rats with an approximate weight of 120–130 g participated in the experiment. The animals were divided into two groups of 30 in each: group 1 was made an incision on the lower jaw under intra-abdominal chloral hydrate anesthesia at a dosage of 350 mg/kg of weight; then a defect in the form of a cavity of 0,5×0,5 mm was formed with a diamond (spherical) boron. The test sample of osteoplastic material was inserted into the formed defect. The wound was tightly sutured with monofilament suture material *Vicril*. The 2nd control group also consisted of 30 rats with similar defects. In the process of regeneration of this group we did not interfere.

## RESULTS AND DISCUSSION

For material production powered bone mineral components and liquid hyaluronic acid solution were used [4]. The paste for filling the bone defect was made according to the developed technological scheme (Fig.1).

To obtain paste consistency, hyaluronic acid gels of various concentrations were used in the work. (1%, 1,5%, 2%). 1% gel had necessary rheological characteristics, stimulated tissue regeneration, accelerated scar resorption [4, 5]. In this regard, 1% hyaluronic acid gel was used in further work. The bone tissue material was placed in 10% neutral formalin and then placed in a decalcifying solution based on Trilon B. then paraffin blocks were made according to the standard histological method both with hematoxylin and eosin staining and according to Van Gieson. Evaluation of microphotographs was made with a microscope LeicaDM 100.

During the experiment, when integrating osteoinductive material, the defect zone was completely replaced by bone tissue, where the main components of the lower jaw bone were determined and represented by compact and spongy bone.

During an experimental morphological study of the biomedical characteristics of the osteoplastic material developed by us, it was found that when introduced into a bone wound, it contributed to the activation of reparative osteogenesis in the area of lower jaw injury in rats, which indicates a successful replacement of the bone defect.

## CONCLUSIONS

1.Bone powder and glucosamine were a matrix for filling the bone pockets with a different number of existing limiting bone walls, as well as for restoring periodontically affected areas of root furcation. Implantation of bone powder along with glucosamine showed high biological compatibility with recipient tissues: practically there was no inflammatory reaction, as well as systemic and local toxicity. During implantation, the normal content of calcium and phosphates in blood serum was maintained due to the supply of calcium ions from bone meal to a specialized bone pool. In addition, hydroxyapatite resorption took place without the formation of a fibrous capsule around the implant.

2. Premix that contained microelements and vitamins induced the speeding up of the reparative osteogenesis.
3. One of the main differences in the technology of

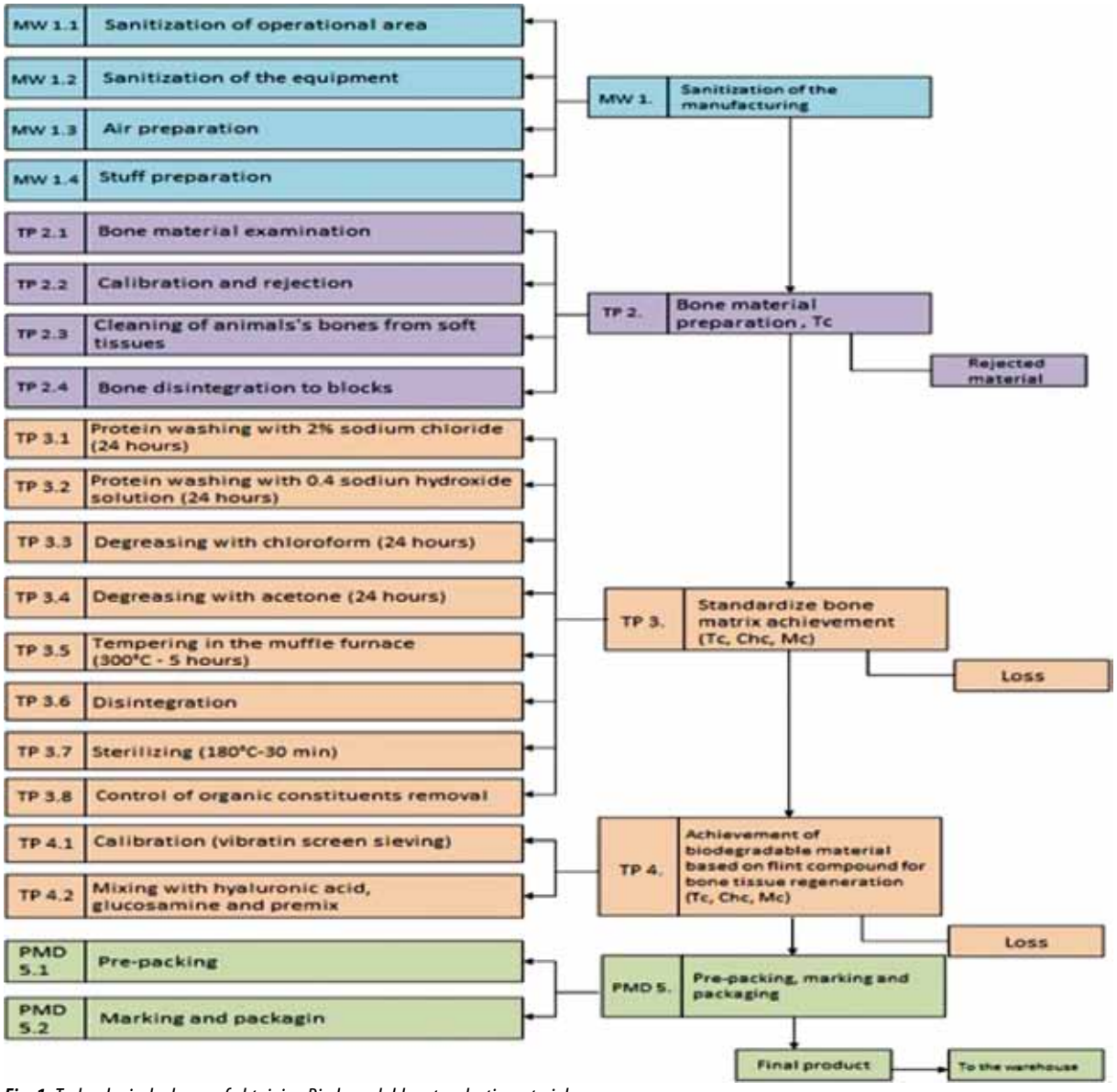


***Fig. 1.*** *Technological scheme of obtaining Biodegradable osteoplastic material for bone tissue regeneration*

***Table 1.*** *Ingredients of biodegradable osteoplastic material*

| Ingredients | Content, % |
|---|---|
| Hyaluronic acid | 10 |
| Bone powder | 75 |
| Glucosamine | 5 |
| Premix* | 15 |

** Premix containing silicon and magnesium oxides, zinc, manganese and copper sulphates, sodium borate and tricalcium phosphate, ascorbic acid, pyridoxine hydrochloride, cholecalciferol*

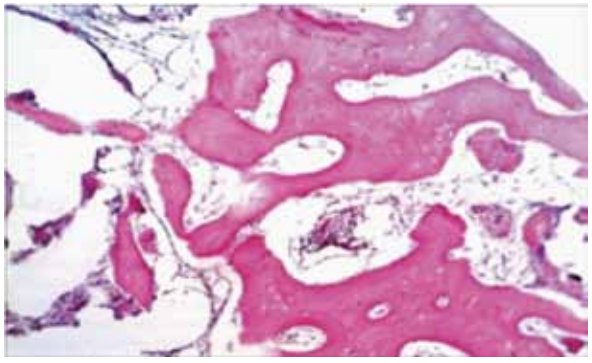

***Fig. 2.*** *Formation of bone tissue after application of osteoplastic material. Staining with hematoxylin and eosin x100*

the developed bioactive osteoplastic preparation was the creation of a slowly metabolized spatially ordered system consisting of the material itself for osteofixation and energetically functional substrates actively involved in the metabolism and formation of bone tissue. All that, in our opinion, creates the possibility of including polysaccharides and vitamins that have a stimulating effect on the growth and proliferation of osteophytes, the formation of a collagen framework.

4. The proposed composition was slowly absorbed by the organism satisfying the local need of organism (in the place of bone tissue damage) for trophic, plastic and energy components.
5. The obtained osteoplastic material had the following properties:

— good tissue tolerance;
— porosity, ensuring bone germination;
— possibility of sterilization without quality changing;
— availability and low price (main components of domestic origin).

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# ANALYSIS OF ORAL FLUID ENZYMES ACTIVITY IN PATIENTS WITH PERIODONTITIS UNDERGOING COMPLEX ANTIBIOTIC THERAPY

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**ABSTRACT** — Antibacterial therapy is the fundamental point in comprehensive treatment of patients with generalized periodontitis. The choice of an antibacterial drug, in view of its maximum effectiveness, is the key to successful pre-surgical preparation of patients with periodontitis to rapid post-surgery rehabilitation and remission maintenance. Our clinical study aims to analyze the activity of oral fluid enzymes (lactate dehydrogenase (LDH), alkaline phosphatase (ALP)) in patients treated with fluoroquinolones. 60 patients were allocated to groups. Each group had a 7-day course of one from four antibiotics: ciprofloxacin (250 mg; 2 times a day); ofloxacin (200 mg; 2 times a day); pefloxacin (400 mg; 2 times a day) and lincomycin hydrochloride (500 mg; 2 times a day). Ciprofloxacin was found to have the highest effect on the LDH and ALP suppression.

**KEYWORDS** — Antibacterial drugs, periodontitis, lactate dehydrogenase (LDH), alkaline phosphatase (ALP), fluoroquinolones, saliva enzymes.

## INTRODUCTION

The pathogenesis, diagnosis and treatment of inflammatory periodontal diseases are an urgent issue faced by dentistry [1–6]. Antibacterial therapy is an integral part of comprehensive treatment offered to patients with generalized periodontitis [7]. Antibiotics of the fluoroquinolone class display a pronounced antimicrobial activity against the major periodontal pathogens, while numerous studies have revealed high activity of these drugs and their potential use when treating periodontitis [8, 9]. In 2002, Yang Q.S. et al. carried out a study showing that fibroblasts of the gum's own layer can accumulate ciprofloxacin, thus functioning as a container that ensures a high extracellular concentration of the antibiotic [10]. Of lincosamides group, the antibacterial drug lincomycin, which has the ability to accumulate in the bone tissue in therapeutic doses and have its effect on flora that is resistant to other antibiotics, has come to be employed widely in periodontics [11].

Most researchers nowadays believe that indicators of mixed saliva are closely related to dental system pathologies. Lactate, for one, is a product of anaerobic microflora metabolism, which is activated through inflammatory processes. In view of this, the activity of lactate dehydrogenase in mixed saliva may be a marker for tissue inflammation in the oral cavity. In case of periodontitis, the proteins of the inflamed tissues undergo proteolysis; an increase in free amino acids leads to an enhanced activity of aminotransferases, which allows making conclusions concerning the severity of the inflammatory process in the oral cavity. Lactate dehydrogenase enzymes and alkaline phosphatase are important indicators in terms of diagnosing destructive processes in case of periodontal disease. This means that studying the composition and properties of mixed saliva is of great diagnostic value, since it allows, in some cases, identifying not just the body status as a whole, yet also that of the oral cavity tissues [12, 13]. Besides, mixed saliva is of interest for diagnostic purposes, since the method of obtaining it is simple, non-invasive, has a minimal cost, and requires minimal processing [14–17].

*Aim of study:*
to analyze the activity of oral fluid enzymes (lactate dehydrogenase and alkaline phosphatase) in patients with periodontitis while employing various antibacterial drugs within comprehensive therapy.

## MATERIALS AND METHODS

The study involved 60 patients (29 males and 31 females) with generalized periodontitis, aged 18 to 50, a mean age of 34 years. The patients were divided into groups (15 persons in each) depending on the antibacterial drug used for the treatment: ciprofloxacin (250 mg; 2 times a day); ofloxacin (200 mg; 2 times a day); pefloxacin (400 mg; 2 times a day) and lincomycin hydrochloride (500 mg; 2 times a day), for 7 days. The control group included 20 people with intact periodontium of the same age.

The object of the study was unstimulated oral fluid (2 ml) obtained through spitting. The activity of the enzymes – lactate dehydrogenase (D. Weissar, 1975) and alkaline phosphatase (W. Kubier, 1973) was identified using a set of chemical reagents and a biochemical analyzer Hospitex (Switzerland).

## RESULTS AND DISCUSSION

To obtain control values, data were obtained on changes in the LD enzymes activity (u/l) in patients with intact periodontium in vitro: intact periodontium 305±7.8; ciprofloxacin 265±7.5; ofloxacin 274±6.7; pefloxacin 281±4.2; lincomycin 280±8.3. Changes in the alkaline phosphatase activity (u/l): intact periodontium 22.6±4.5; ciprofloxacin 14±1.1; ofloxacin 16±3.3; pefloxacin 18±3.1; lincomycin 19±2.1. An analysis of the outcomes revealed a decrease in the lactate dehydrogenase and alkaline phosphatase activity after using the antibiotics in vitro, and the following pattern could be observed: ciprofloxacin demonstrated the highest degree of suppression, whereas the same index was the lowest in lincomycin.

Prior to the treatment, patients with generalized periodontitis had an LDH activity (u/l) of 378±12.4, which exceeds significantly that of intact periodontitis; after a course of treatment with an antibacterial drug (Day 7) the following values were obtained: for ciprofloxacin 298±5.2; ofloxacin 301±7.3; pefloxacin 318±4.2; lincomycin, 323±6.2.

A similar trend was observed when studying the alkaline phosphatase activity (u/l): before treatment, 75±8.3; Day 7 into the treatment with ciprofloxacin 23.2±3.6; ofloxacin 24.1±4.2; pefloxacin 26.2± 3.3; lincomycin 38±5.3.

The antibacterial drugs, therefore, could be arranged as follows in the order of a decreased inhibitory capacity (with respect to the oral fluid enzymes activity): ciprofloxacin, ofloxacin, pefloxacin, lincomycin.

The studies also revealed a change in the Michaelis constant (Km) of biochemical responses catalyzed by LDH and AP in patients with generalized periodontitis. LDH Km (M) before the treatment was 1.37 ± 0.1, while after the treatment (Day 7 of the study) for ciprofloxacin it was 2.51±0.1; for ofloxacin, 2.38±0.11; pefloxacin, 2.12±0.18 and for lincomycin, 1.94± 0.16. AP Km (M): before the treatment, 2.01±0.13; 7 days into the treatment: for ciprofloxacin, 3.5±0.13; for ofloxacin, 3.38±0.14; pefloxacin, 3.24±0.11; lincomycin, 3.11±0.09.

Given the above, there has been an increase in Km for LDH and AP observed after treatment with antibacterial agents, which points at a decrease in the enzyme activity, which can be regarded as a direct inhibitory effect that antibacterial drugs have on LDH and ALP (this effect was detected in vitro). As far as the degree of effect on Km is concerned, the drugs can be arranged in the following sequence: ciprofloxacin, ofloxacin, pefloxacin, lincomycin.

The obtained data indicated that ciprofloxacin containing cyclopropyl in the quinolone cycle has a more pronounced inhibitory effect on the bacterial flora, which, respectively, led to a decreased LDH and alkaline phosphatase activity in the oral fluid. This can be accounted for by suppressed bacterial growth against fluoroquinolone agents due to their lipophilicity, i.e. the capacity to dissolve in the bacterial cell membranes, and, consequently, to better penetrate into the cells of microorganisms. Pefloxacin, which contains only one ethyl group, revealed the lowest inhibitory effect on the oral fluid enzyme activity.

## CONCLUSION

The clinical and laboratory studies have demonstrated that ciprofloxacin offers the highest effect on the LDH and alkaline phosphatase suppression in the oral fluid of patients with generalized periodontitis. Therefore, this finding can be employed when selecting a medicine for antibacterial treatment.

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# ORTHOPEDIC TREATMENT FOR MASTICATORY MUSCLES PARAFUNCTION: EXPLANATION BASED ON CLINICAL AND FUNCTIONAL STUDY

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**ABSTRACT** — This study was conducted to investigate two clinical types of masticatory muscles parafunction: teeth compression and teeth grinding using clinical and functional methods. Teeth compression and teeth grinding were found to have a common tooth compression symptom. Our finding has been proven not only through clinical, yet via electromyographic and axiographic research methods as well. Based on the obtained data we developed a palatal plate-occlusal guard. It enables to fix reliably the lower jaw and reduces muscle tension. The proposed appliance helps prevent possible complications that occur in the course of orthopedic treatment.

**KEYWORDS** — masticatory muscles parafunction, electromyographic study, electronic axiography, palatal plate, occlusal guard.

## INTRODUCTION

Implantation is an advanced method employed for selecting dental treatment when replacing missing teeth. Indications for the methods are expanding steadily. Certain pathological conditions affecting the patient's dental status may remain hidden and require a more detailed examination [12–16].

Such disorders include masticatory muscles parafunction. As reported by the WHO, the prevalence of this problem lies in a range from 10% to 27% for various age groups [1]. It is postulated that masticatory muscles parafunction represent a function, which is unconscious, impractical and related to neither chewing nor speech activities, and which manifests itself as spontaneous lower jaw movements and unnecessary teeth compression. Unidentified parafunction results in excessive load not only on the teeth, yet on the implants, too, thus contributing to further progress of serious complications [2, 7, 8, 10, 11]. The most common clinical parafunctions include teeth clenching and grinding of teeth (bruxism). These share the following symptoms: chewing muscles tension and spasm; habitual teeth clenching, which leads to their erasure and chipped enamel. As far as available literature is concerned, we failed to find sufficient information regarding the nature of teeth compression and grinding, their combined action and alternation. The available study outcomes that describe masticatory muscles bioelectric activity caused by surface electromyography, are largely contradictory and require further investigation. Patients suffering from respective symptoms are recommended to test their dental status and undergo a comprehensive examination [3–5, 17–19] followed by some special preliminary orthopedic measures [6, 9].

*Aim of the study* was to improve effectiveness of orthopedic treatment offered for cases involving masticatory muscles parafunction, as well as its clinical and functional explanation.

## MATERIALS AND METHODS

We examined 59 patients aged 28 through 65 years. The inclusion criteria were complaints of teeth grinding, teeth abrasion, masticatory muscles fatigue, and difficulty opening the mouth due to spasm in the masticatory muscles. The exclusion criterion was destructive disorders of temporomandibular joint (TMJ). The patients were divided into 2 groups subject according to the registered complaints: Group I — patients with the clinical type of *teeth compression* parafunction (23 persons); Group II — patients with the clinical form of *teeth grinding* parafunction (22 persons). The control group included persons with neither dental nor somatic pathology (14 persons), while it was compatible the major groups in terms of the age and gender criteria. The study involved clinical methods as well as comprehensive laboratory and instrumental research: model analysis in the Stratos 300 articulator (Ivoclar); electronic axiography (Arcus digma II, Kavo); masticatory muscles electromyography (Synapsis, Neurotech). The obtained data underwent statistical analysis using the SPSS 25 software package where the values of the arithmetic mean (M) and standard deviation (G) standard error (m) were calculated. The differences between the samples were evaluated through the Student's t-test and the Mann-Whitney-Wilcoxon U-test with the Pearson $\chi^2$ test employed; the differences were considered statistically significant at a probability level of 95% ($p<0.05$).

## RESULTS AND DISCUSSION

Table 1 offers the outcomes of the clinical examination.

No statistically significant differences were identified between the groups based on the $\chi^2$ criterion.

The tension and pain index of the masticatory muscles in Group I was 2.12±0.03, in Group II — 2.67±0.01. The tension and pain index of the temporal muscle was 1.53±0.06 and 1.77±0.03, respectively.

***Table 1.*** *Clinical examination, prior to the treatment*

| Symptoms | Group 1; n=23 | | Group 2; n=22 | |
|---|---|---|---|---|
| | Males, n=14 | Females, n=9 | Males, n=15 | Females, n=7 |
| Masticatory muscles spasm | 14 | 9 | 15 | 7 |
| Teeth grinding | 5 | 1 | 15 | 7 |
| Pain in masticatory muscles | 14 | 8 | 15 | 7 |
| Masticatory muscles fatigue | 14 | 9 | 15 | 7 |
| Deviation, deflection | 3 | 2 | 15 | 7 |
| Super-contact | 12 | 8 | 15 | 7 |
| Disturbed mouth opening | 4 | 2 | 15 | 7 |
| Reducing height of the lower face | 11 | 6 | 15 | 7 |

The values of the masticatory muscle stress index in Group II proved to exceed that of Group II by 8.4%, which could be due to the fact that the patients of Group I featured no nocturnal teeth compression. The temporal muscle tension index of Groups I and II differed slightly, yet but if matched against the masticatory muscle itself, then the difference with Group I was 72.2%, and Group II — 66.3%, which reveals a stronger tension the masticatory muscle had to experience.

Table 2 offers a view at the electromyography outcomes.

As can be seen from the table, almost all EMG indicators featured statistically significant differences in Groups I and II (except for Sc Td/Ts, Sc Md/Ms). This is also true for the angular parameters of the sagittal joint path, but not for the incisal point trajectory between Groups I and II.

Treating masticatory muscles parafunction is one of the most challenging issues since the disease is associated with a disturbed function of the neuromuscular complex. To relax the masticatory muscles, we developed a special palatal plate-occlusal guard (RF patent for utility model # 182370 of 15/08/2018). The palatal plate is to be installed on the upper jaw, and is made from a rigid base plastic with occlusal patches all over the upper and lower dentition, and tooth prints on the occlusal surface. The vestibular side of the mouthpiece bears pelottes that overlap the upper and lower jaw teeth to the equator level. At the same time, it implies separation of dentition up to 3.5 mm. Then, additionally, an elastic sandwich-shaped piece of plastic is applied to the occlusal surface of the plate, facing the lower jaw teeth and pelottes. That serves as a soft pad, whereas the dentition separation is to be up to 8.0 mm already. The soft pad acts as a shock absorber in case there is teeth compression.

Following the treatment, the differences between Groups I and II were almost gone. There were some statistical differences to remain for Td A and for Ts A (μV) only. Other electromyography and axiography values featured basically no difference from the statistical point of view (p>0.05). Both groups' values approached those of the control group.

## CONCLUSION

The clinical and functional methods employed for studying the masticatory muscles parafunction revealed the predominance of the masticatory muscles indicators compared to the temporal muscles, which was observed through a lower value of the K coefficient. The proposed palatal plate-occlusal guard causes a positive effect by relieving tension in the masticatory muscles and fixing reliably the lower jaw over the treatment period, thus, shortening the length of treatment.

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***Table 2.*** *EMG and electronic axiography values at maximum jaw compression in case of conventional occlusion, prior to treatment*

| EMG values | Group I, n=23 | Group II, n=20 | Control group n=14 | p1-2 | p1-к | p2-к |
|---|---|---|---|---|---|---|
| Td A (μV) | 679.6±61.2 | 618.4±59.4 | 477.4±45.7 | 0.001 | <0.001 | <0.001 |
| Ts A (μV) | 664.8±63.4 | 601.2±57.3 | 465.1±41.9 | 0.001 | <0.001 | <0.001 |
| Md A (μV) | 698.4±67.2 | 641.3±63.7 | 513.7±47.4 | 0.006 | <0.001 | <0.001 |
| Ms A (μV) | 702.7±69.8 | 638.9±62.9 | 486.6±44.8 | 0.002 | <0.001 | <0.001 |
| Sc Td/Ts | 0.72 ±0.1 | 0.68 ±0.2 | 0.98 ±0.2 | 0.398 | <0.001 | <0.001 |
| Sc Md/Ms | 0.67 ±0.2 | 0.64 ±0.1 | 0.95 ±0.2 | 0.531 | <0.001 | <0.001 |
| Sc Td/Md | 0.74 ±0.2 | 0.61 ±0.1 | 0.89 ±0.2 | 0.009 | 0.016 | <0.001 |
| Sc Ts/Ms | 0.76 ±0.1 | 0.63 ±0.2 | 0.91 ±0.1 | 0.008 | <0.001 | <0.001 |
| Sagittal path angle, right | 49.5±4.6 | 52.4±4.9 | 43.2±3.3 | 0.047 | <0.001 | <0.001 |
| Sagittal path angle, left | 50.0±4.8 | 53.8±5.1 | 44.1±3.6 | 0.014 | <0.001 | <0.001 |
| Incisal point trajectory | 30.6±2.5 | 31.2±2.5 | 40.2±3.1 | 0.425 | <0.001 | <0.001 |

***Note:*** *Td – right temporal muscle; Ts — left temporal muscle; Md — right proper masticatory muscle; Ms — left proper masticatory muscle; Sc — symmetry coefficient.*

***Table 3.*** *EMG and electronic axiography values at maximum jaw compression in case of conventional occlusion, after treatment*

| EMG values | Group I, n=23 | Group II, n=20 | Control group n=14 | p1-2 | p1-к | p2-к |
|---|---|---|---|---|---|---|
| Td A (μV) | 550,3±51,6 | 513,6±48,5 | 477,4±45,7 | 0,018 | <0,001 | 0,014 |
| Ts A (μV) | 537,2±50,8 | 504,9±47,9 | 465,1±41,9 | 0,034 | <0,001 | 0,005 |
| Md A (μV) | 589,2±53,3 | 561,2±52,2 | 513,7±47,4 | 0,082 | <0,001 | 0,003 |
| Ms A (μV) | 594,1±53,9 | 571,1±52,9 | 486,6±44,8 | 0,156 | <0,001 | <0,001 |
| Sc Td/Ts | 0,89 ±0,2 | 0,87 ±0,1 | 0,98 ±0,2 | 0,676 | 0,139 | 0,023 |
| Sc Md/Ms | 0,84 ±0,2 | 0,83 ±0,2 | 0,95 ±0,2 | 0,868 | 0,072 | 0,051 |
| Sc Td/Md | 0,81 ±0,1 | 0,79 ±0,1 | 0,89 ±0,2 | 0,506 | 0,095 | 0,038 |
| Sc Ts/Ms | 0,82 ±0,2 | 0,90 ±0,2 | 0,91 ±0,1 | 0,187 | 0,065 | 0,834 |
| Sagittal path angle, right | 45,6±3,9 | 47,8±4,7 | 43,2±3,3 | 0,094 | 0,031 | <0,001 |
| Sagittal path angle, left | 46,1±4,3 | 48,3±3,1 | 44,1±3,6 | 0,056 | 0,099 | <0,001 |
| Incisal point trajectory | 39,3±3,0 | 38,8±2,9 | 40,2±3,1 | 0,573 | 0,328 | 0,125 |

***Note:*** *Td — right temporal muscle; Ts — left temporal muscle; Md — right proper masticatory muscle; Ms — left proper masticatory muscle; Sc — symmetry coefficient.*


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# HISTOMORPHOMETRIC PARAMETERS IN SIMULATED GINGIVAL RECESSION

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**ABSTRACT** — This study addresses the nature of morphological changes occurring in periodontal tissues through the development of gum recession in the experiment. Gingival recession was found to occur along with a decrease in epithelial thickness of the mucous membrane as well as a decreasing cross-sectional area of blood vessels, whereas the connective tissue featured fibrinoid swelling. Initial tissue ischemia in the affected area can have a negative effect on reparation. This should be borne in mind when planning a periodontal plastic surgery.

**KEYWORDS** — gum recession, microcirculation, mucogingival plastic surgery, periodontology.

## INTRODUCTION

To date, one of the current challenges faced by periodontology is to improve the treatment of gum recession. This is viewed not only as a medical problem but also an aesthetic one [1–13]. Various authors note that the prevalence of gum recession ranges from 40 to 100%, while it is important to keep in mind that the disease prevalence may depend on various subjective factors leading to different interpretations of research outcomes [14]. In case there are no contraindications, surgical intervention stands as the optimal method for treating gum recession, and it is important to bear in mind that surgery planning has to be done in view of the initial status of the tissues at the potential intervention area [15, 16].

In view of the above, relevant is studying the details of knowledge regarding the nature of morphological changes in periodontal tissues through the development of gum recession within the experiment.

*Aim of study:*
to study the histomorphometric parameters of the rat oral mucosa in experimental gingival recession.

## MATERIALS AND METHODS

Our study included 40 male Wistar rats weighting 180–220 g aged 3,5–4 months. 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. The animals were fully anesthetized prior to surgical procedures.

To better understand the nature of morphological changes occurring in tissues during gingival recession, the experimental animals were divided into 2 groups. Group 1 included 20 animals whose oral mucosa remained unchanged. Group 2 included 20 animals that had gum recession simulated surgically via an experiment. Animals with gum recession were removed from the experiment 28 days after the surgery. Two fragments of oral mucosa were obtained from each animal in each group. The animals that had undergone surgery, had their fragments obtained from the zone that was — visually — the most altered. Following a standard diagnostics procedure, three micro-preparations were obtained from each fragment. Then, 5 microphotographs were obtained from each micro-preparation, to be further examined employing morphometric methods. The results were processed using the "Statistica version 8.0" software.

## RESULTS AND DISCUSSION

When studying the micro-preparations from intact animals, whose oral mucosa remained unchanged, the following results were obtained: no inflammatory infiltration detected; the number of connective tissue cells (fibroblasts and fibrocytes) was 75.8±2.5 cells within vision. The relative area of connective tissue was 82.2±3.4%, i.e. connective tissue accounted for the largest volume of soft gum tissue. The cross-sectional area of blood vessels was 12.5±0.7% whereas the relative area of muscle fibers proved to have the smallest value, 5.8±2.3%. The intact epithelial layer thickness in the rat oral cavity averaged 25.3±1.2 microns.

The experimental group of animals that had gum recession induced in them, featured no inflammatory

infiltration. The number of connective tissue cells decreased compared to the first group. The connective tissue area increased with fibers reaching 86.2±4.3%. There were some changes in the connective tissue thickness to be observed appearing as dissociated and chaotic arrangement of fibers, and accumulation of amorphous matter, which presented signs of fibrinoid swelling. The cross-sectional area of blood vessels went down to 7.1±1.6%. The muscle component area decreased to 4.4±2.2%. The epithelial layer thickness decreased significantly due to reduced nutrition (down to 11.2±0.8 microns). It should be noted that this type of change is typical of dystrophic processes, which offers another proof to the nature of gum recession development.

## CONCLUSION

Thus, the study lead us to conclude that a surgery on receded gingiva is performed in the condition of impaired microcirculation at the affected site and, consequently, dystrophic changes in the tissue. Initial tissue ischemia in the affected area can have a negative effect on reparation. This should be borne in mind when planning a mucogingival plastic surgery.

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# QUALITY OF LIFE IN GERIATRIC PATIENTS WITH VARIOUS DENTITION DEFECTS

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**ABSTRACT** — Subject: Population aging is a trend in modern society and elderly people who have gained social and professional status claim a significant position in it. Factors, such as dissatisfaction with dental appearance, confusion when smiling or talking, chewing and speech disorders, significantly complicate a person's daily activities and negatively affect self-confidence and social behavior. The need for social reforming to extend working activity has become an inevitable result of population aging. The objective of this research was outlined by the need to improve the quality of life in elderly patients, to extend social longevity.
This paper proposed provides information characterizing the structure of dental orthopedic morbidity in geriatric patients. The authors describe the results of their own research to identify the relationship between life quality indicators and dentition defect types in the group of patients under study.
Methodology: The research involved an observational longitudinal prospective study with a double survey of respondents. An alternative version of the OHIP 14 questionnaire was chosen as a tool for studying the life quality.
Results: It was found that dentition defects have a significant impact on the life quality of elderly populations
Conclusion: Studying the structure of dental orthopedic morbidity can optimize provision of dental orthopedic care, improve life quality and prolong socialization of seniors.

**KEYWORDS** — geriatric dentistry, socialization, life quality.

## INTRODUCTION

Over the past decades, interest in scientific research focused on studying the criteria for assessing the patients' life quality has significantly grown in modern science. As a result of the established global trend of natural aging of the population, social adaptation of elderly populations is becoming an urgent issue of modern scientific and practical medicine. The need for social reforming to extend working activity has become an inevitable result of population aging. Elderly people who have gained social and professional experience claim a significant position in modern society. Factors that may affect quality of life (dissatisfaction with dental appearance, confusion when smiling or talking, speech defects, inability to eat favorite food, distinguish nuances of taste) significantly complicate daily activities and negatively affect self-confidence and social behavior [1–4].

Dental orthopedic rehabilitation of patients in the older age group has a number of features. It is in them that dystrophic forms of dental diseases caused by the development of involutive processes of an aging organism, prevail over inflammatory forms. Another significant feature of gerontodentistry is polymorbidity – the presence of two or more long-term chronic somatic diseases in a patient manifesting itself in the mouths of the elderly. All this significantly impairs the quality of their life [5–11].

The data cited in the literature indicate 100% of the need in qualified dental orthopedic care on the part of the elderly. The need for single artificial crowns and post structures first occurs at the age of 19–30, reaches its maximum at 41–50, and by the age of 60 and older it becomes insignificant in the general structure of orthopedic fixtures. The need for dental orthopedic treatment with bridgework first appears at the age of 19–30, reaches its maximum at 40–50, and gradually decreases towards the age of 60 and older. The need for dental orthopedic treatment with removable dentures first appears at the age of 40–49. This characteristics is minimum in the age group below 50, gradually increasing and reaching a maximum in the age group of 70 years and older [12, 13].

Life quality (LQ) of older people is largely determined by dental health [14–29]. These are high prevalence rates of dental orthopedic morbidity in elderly populations, the need for medical and social rehabilitation in patients of this age group that determine the *relevance* of this paper. A systematic and multifaceted approach to the study of this problem may underlie the solution to the issues of organizing dental orthopedic care for this population group in connection with the socially significant social and labor reforms being consistently implemented in Russia and Europe.

*Research objective:*
to determine the structure of dental morbidity in the elderly patients and identify possible relationships between their life quality and dentition defect types.

## MATERIAL AND METHODS

Standard international methodology for studying the life quality begins with drawing up a research protocol defining the goals and objectives of scientific work, criteria for including patients, calculating the minimum sample size and designing a study of the life quality in the elderly, which was defined as a classic observational longitudinal prospective study with a double survey of respondents.

An alternative version of the specialized questionnaire THE ORAL HEALTH IMPACT PROFILE, OHIP 14, was chosen as an *optimal tool* for studying the life quality of dental patients aged 60 and older.

The study group included 1,000 geriatric patients divided into three groups[1]. The *Elderly People* group of was formed by 408 dental patients aged 60–74, which amounted to 40.8%±0.44. The *Old People* group included 481 patients aged 75–89 years, which amounted to 48.1%±0.87. *Long-livers* — patients aged 90 and older — constituted the smallest group of 111 people, 11.1%±0.83 (Fig. 1).

In our study group 573 women (57.3%±0.83) slightly prevailed over 427 men (42.7%±0.87) (Fig. 2).

Completion of the primary medical documentation which includes informed voluntary consent to personal data processing, medical diagnostic and therapeutic measures, and participation in a scientific experiment, followed by a dental medical examination, filling out a clinical card and a specialized questionnaire for studying life quality.

Dental orthopedic treatment was provided within the required scope during the second and subsequent visits.

The total number of orthopedic fixtures manufactured and installed was 9,843 pcs., of which 3,016 were pin-type, 3,336 artificial crowns, 1,410 bridges, 957 removable plate dentures with partial loss of teeth, 404 clasp dentures, 720 removable dentures with total loss of teeth (Fig. 3).

Four months after the end of dental treatment, the patients were asked to re-fill a specialized questionnaire for studying life quality. Repeated questioning involved a remote survey.

The resulting data were analyzed by standard methods of statistical processing using PC software: MicrosoftExcel and Statistica 6.0.

## RESULTS

All examined patients were divided into groups based on the Kennedy (1923) classification of dentition defects convenient to use as a descriptive tool that displays dentition defects in detail. The number of patients with complete loss of teeth was calculated separately.

The structure of dental morbidity in the elderly populations admitted to a municipal dental orthopedic care indicates the following: out of 1,000 people - 120 people (12%±1.2) had bilateral terminal dentition defects, 260 people (26%±1.3) — unilateral terminal defects, 200 people (20%±1.3) — included defects in the lateral dentition parts, 60 people (6%±1.5) — defects in the anterior dentition parts, complete absence of teeth was observed in 360 people (36%±1.2).

A pattern was revealed when studying life quality of the examined patients. The nature of the dentition defect was important. The responses of patients with bilateral terminal defects (36.96±1.7), unilateral terminal defects (37.10±1.5) and included defects of the lateral dentition parts (37.09±1.7) have not shown statistically significant differences in comparison with each other. All patients noted poor life quality associated with a loss of taste for food, pain, as well as difficulty in eating, the need for selective choice of food, and interruption of eating. Elderly people noted irritability, difficulties in work and rest, they said that from time to time their life becomes uninteresting, and they are forced to *drop out* of it, refusing to socialize.

Patients with defects in the anterior dentition part more often noted social inconvenience, awkwardness and confusion when communicating with people, difficulty in pronouncing words, saliva splashing, a tight smile and lack of laughter. In general, their life quality is somewhat better (34.54±1.34) in comparison with patients with defects in the lateral dentition parts.

Patients with complete loss of teeth often noted a lack of taste in once favorite foods, the need to choose food due to missing teeth and difficulties in chewing food, interruption or premature completion of eating due to pain in the mouth and chafing. All subjects reported inconvenience, awkwardness and confusion when communicating with people, difficulties in pronouncing words, saliva splashing, tight smile and lack of laughter. They pointed out increased irritability and difficulties in everyday work and rest. They noted that their life has become less interesting and sometimes they completely *drop out* of it, denying themselves to communicate with friends and relatives. Life quality of patients in this group was rated as the lowest (42.12±1.92).

Four to six months after orthopedic dental rehabilitation a second survey followed to study life quality of the elderly patients who had managed to get used to new orthopedic dental fixtures.

All patients began to notice taste loss less often, had less mouth pain and chewing difficulties. The re-

[1] *Age classification of the late period of human life (WHO, 1963) 45–59 — average age; 60–74 — old age; 75–89 — senile age; 90 and older — longevity.*

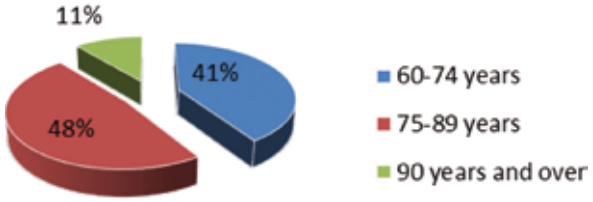


*Fig. 1. Age structure of the respondents; (%)*

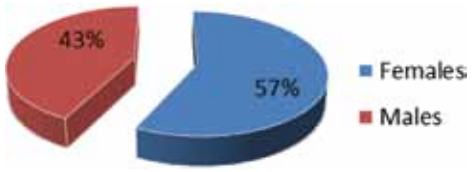


*Figure 2. Gender composition of the respondents; (%)*

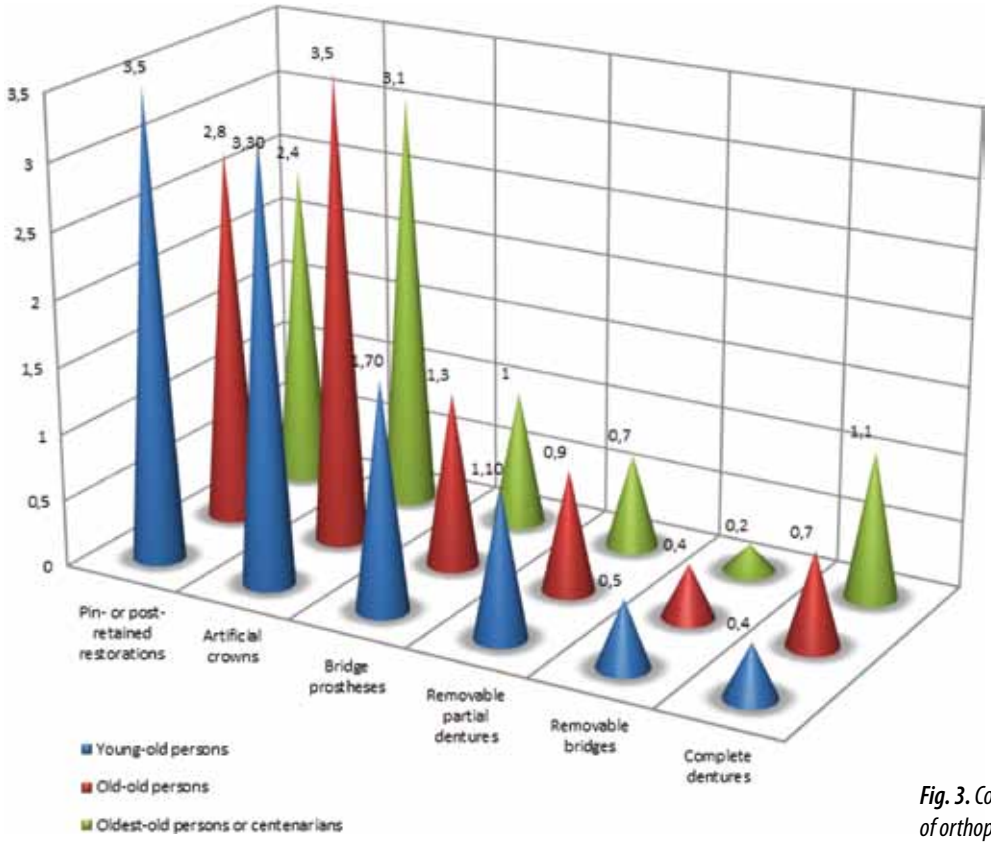


*Fig. 3. Comparative analysis of orthopedic fixtures*

spondents began eating better and interrupting meals less often. Less irritation and difficulties in everyday life and more confidence were reported. The life of the seniors has become more interesting and diverse.

Life quality of geriatric patients has changed after dental orthopedic treatment. Patients with bilateral terminal defects have shown improvements from 36.96±1.7 to 25.35±1.17, Δ11.61, with unilateral terminal defects — from 37.10±1.5 to 25.64±1.15, Δ11.46, and with included defects in the lateral dentition parts — from 37.09±1.7 to 25.35±1.17, Δ11.74. Life quality of patients with the anterior dentition defects improved from 34.54±1.34 to 23.09±1.45, Δ11.45. The worst life quality indicators were shown by patients with complete loss of teeth from 42.12±1.92 to 30.58±1.51, Δ11.54.

It should be noted that in patients with the lateral dentition defects, functional indicators of the questionnaire responded more actively to changes in life quality, in patients with the anterior dentition defects it were social indicators, in patients with complete absence of teeth — both functional and social indicators of the questionnaire equally evidenced changes in life quality.

After the dental orthopedic treatment, life quality in all our patients has significantly improved, as evidenced by the structure of OHIP-14 responses, namely, an increase in *never* responses from 4.94% to

22.70%, an increase in *almost never* responses from 6.42% to 42.24%, a significant decrease in "very often" responses from 34.36% to 6.46%. The total score of the questionnaire decreased by an average of 30.7%.

*Conclusions:*

life quality of elderly people is influenced by the presence and type of dentition defect. Studying the structure of dental orthopedic morbidity can optimize the quality of dental orthopedic care, improve life quality, and prolong social longevity.

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*Conflict of interests:*

The authors claim that there is no conflict of interests.

*Statement of ethics*

According to the international rules for clinical trials set out in the Helsinki Declaration of the World Medical Association (1975), and the international Draft Guidelines on Good Clinical Practice recommended by WHO (1956), as well as the clinical practice rules of the Russian Federation (order of the RF Ministry of Health dated 01/04/2016 No. 200n), protection of human rights was ensured in clinical trials with the participation of people. The protocol of this study was approved by the local ethics committee (protocol No. 5 dated 20/05/2016). Biomedical examination involved highly qualified trained personnel using certified equipment.

*Consent to participate:*

all patients who participated in the study filled out informed voluntary consent to personal data processing, medical diagnostic and therapeutic measures, and participation in a scientific experiment.

*Availability of data and materials:*

this work is a continuation of previous research.

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# MODIFICATION OF THE DENTAL ARCH SHAPE USING GRAPHIC REPRODUCTION METHOD AND ITS CLINICAL EFFECTIVENESS IN PATIENTS WITH OCCLUSION ANOMALIES

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**ABSTRACT** — Based on the results of the dentoalveolar system morphometry performed in patients with permanent teeth physiological occlusion we have developed a method for studying the anterior dental arch. It follows the circle of geometry patterns as well as stable values in the medial-distal dimensions of the front teeth crowns. In view of the mesial-distal dimensions of 14 teeth and dental arch width between the second molars and individually built radius of the circle we modified the method of dental arches graphic reproduction. The first stage of the dental arch individual shape graphic reproduction implies designing a dental pentagon, whereas its base is the width of the dental arch between the second molars, and the median sagittal line determines the depth of the dental arch. The upper sides of the pentagon (incisor-canine diagonals) run from the central interincisal point to the canine point, while the lower sides (canine-molar diagonals) connect the canine points to the molar points. At the second stage of the dental arch individual shape graphic reproduction, a circle is outlined, whose radius is related directly to the width of the anterior dental arch, and has an inverse relationship with its depth. There is a proof offered for clinical feasibility of the method employed to predict the optimal individual shape of the dental arch through graphic reproduction in patients with class I Angel occlusion issues. The study showed that the effectiveness of therapeutic and diagnostic measures for patients with abnormal shape and size of dental arches. It can be achieved if the sequence of the graphic construction stages is strictly followed.

**KEYWORDS** — graphic reproduction of dental arches, individual shape of the dental arch, occlusion anomalies, odontometry, gnathic type of dental arch, dental type of dental arch.

## INTRODUCTION

Solving the problems of modern orthodontics viewed as a complex dental discipline, is aimed not at correcting the teeth position, dentition and bite alone, yet also at creating conditions to ensure proper growth of the jaw bones, correcting the shape of the facial skull, improving the dental apparatus functions, and restoring the facial aesthetics [2, 6, 8, 12, 24, 28, 41, 46].

The traditional methods that allow in most cases achieving optimal outcomes in correcting dental anomalies and deformities at the early stages still maintain their clinical value. However, given the progress of medical technologies and knowledge, there have appeared a significant number of advanced methods and means of treatment, which allow carrying out high-level treatment, as well as taking preventive measures, working with patients who need orthodontic assistance [13, 21, 26, 32, 44, 47–51].

Optimal functioning of the dentoalveolar apparatus in case of an orthognathic bite, taken as a type of physiological bite, is achieved through the best aesthetic and morphological optimum, the highest indicators of the chewing function, favorable conditions for somatic swallowing, as well as a comprehensive tongue function. Well-developed signs of physiological occlusion, which are the result of the normal teeth position, the closing of the teeth-antagonist pairs (dental ridges), a well-shaped occlusal plane, with the coordination and control from the central nervous system, will ensure a balanced performance of the temporomandibular joint, the maxillofacial muscles, and the biomechanic features of the lower jaw movement [5, 16, 19, 29, 38, 40, 43].

One of the most urgent issues that orthodontists have to face currently, implies improving the diagnostics of dental anomalies, since this is important in terms of setting diagnoses, selecting the treatment tactics, and identifying the scope of the measures to be taken. No other dental discipline has the identification of an anomaly — and therefore the identification of the treatment aims — as significant as in orthodontics, so there is every reason for diagnostic research to be considered a

key factor at the initial stages of orthodontic care [1, 4, 7, 14, 17, 23, 25, 39].

Specialists have proposed a large number of special examination methods (clinical, cephalometric, anthropometric, X-ray, functional), which, taken together, allow obtaining a fairly complete and objective picture of the dental system status, as well as assessing properly the morphofunctional changes that occur due to various types of anomalies in different age groups [3, 9–11, 15, 20, 22, 30, 33–36].

The construction of a dental arch employing a geometric and graphical method has stirred interest from researchers for many decades. The proposed graphic reproduction of the dental arch by Howley-Herber-Herbst method has already entered both academic and special literature, to turn into an attribute for diagnosing occlusion anomalies. Clinicians claim that the effectiveness of the dental arch graphical construction by the Howley-Herber-Herbst method has proven itself in cases where the width of the dental arches between the canines is twice the depth of the anterior dental arch [27, 31, 37, 42, 45].

There is special attention to be paid to the graphic construction of the dental arch, based on the circle geometry regularities. The arch radius is calculated based on the central angle value. The depth and the width of the anterior segment are to be calculated through the Huygens formula, where the length of the dental arch is taken as the sum of the width of the six front teeth crowns. This method allows calculating the dental arch latitudinal indicators in the canine area, the depth of the anterior segment in case of dental arches anomalies, taking into account odontometric values [18]. There has been a method developed for constructing an arch, where the circle diameter is viewed as a value equal to the difference between the width and the depth of the dental arch to the level of the canine location. This graphic design, however, applies to patients with permanent teeth physiological occlusion [52, 53].

Despite the numerous methods of dental arch graphic reproductions available nowadays, there is no data concerning the methods of constructing dental arches for occlusion anomalies, taking into account the individual features of the dental system, which is the rationale behind, and the purpose, of this study.

*Aim of study:*

to improve the dental arches graphic reproduction method in patients with occlusion anomalies taking into account individual specifics of the dental system.

## MATERIALS AND METHODS

When dealing with patients featuring abnormal shape and size of the dental arches, measuring the linear parameters proves complicated due to the abnormal teeth location in the front and side parts. Modification of the dental arches graphic reproduction method is based on employing stable biometric indicators in the transversal direction along with an evaluation of the inter-canine transversal and the depth of the dental arch anterior part. The radius of the circle for the front teeth location was calculated following the formula below:

$$R = \frac{\left(\frac{W_{(c\text{-}c)}}{2}\right)^2 + \left(D_{(in\text{-}c')}\right)^2}{2 \cdot \left(D_{(in\text{-}c')}\right)}$$

where R is the circle radius; W(c-c) is the front arch width; D(in-c') is the anterior arch depth. The transversal measurements of the anterior part were performed using the Pont and Korkhaus methods, where the arch was divided into segments through the Pont points at the first premolars (Fig. 1).

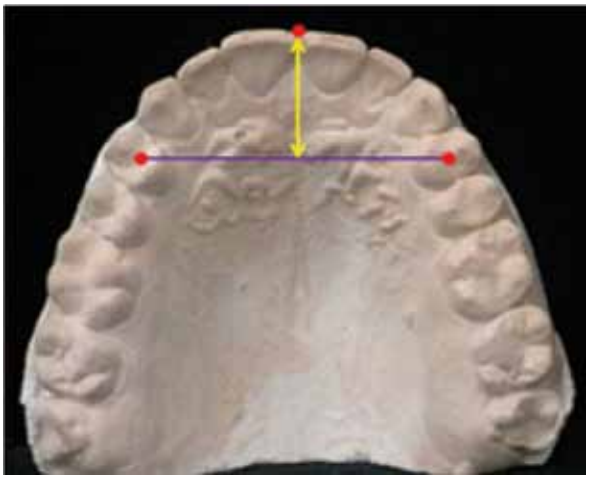


***Fig. 1.*** *Dental arch depth measurement*

The algorithm for constructing a graphic reproduction of the dental arch included the following calculation and diagnostic stages. First, a dental (incisor-canine-molar) pentagon was designed based on dental arches biometric indicators. On the dental arch, the location of the central incisal point was marked as *in* (*incisivus*); the canine points were marked with *c* (*caninus*), located on the canines tearing tubercles. The points located on the distal tubercles tops on the vestibular side of the permanent bite second molars were marked as *m* (*molares*). The canine and the molar points were connected with conventional lines that determined the transversal dimensions of the arches

(inter-canine and intermolar distance). The middle of these lines was marked with *c′* and *m′*. From the *m′* point, a perpendicular was drawn, which ran through the *c′* and *in* points to divide the arch into symmetrical parts. In the sagittal direction, the depth of the dental arch anterior part was identified as the distance between the *in-c′* points. The depth of the complete dental arch corresponded to the *in-m′* (Fig. 2).

which, according to Bolton, falls within the normal value of the *anterior ratio* index (77.28%). The total value of the mesiodistal sizes for 12 teeth in the upper jaw was 97.56 mm; for the lower jaw — 89.06 mm; the overall ratio index (by Bolton) was 91.29%, which corresponds to the norm and indicates compliance with the odontometric indicators. The length of the maxillary dental arch was 117.06 mm, whereas the

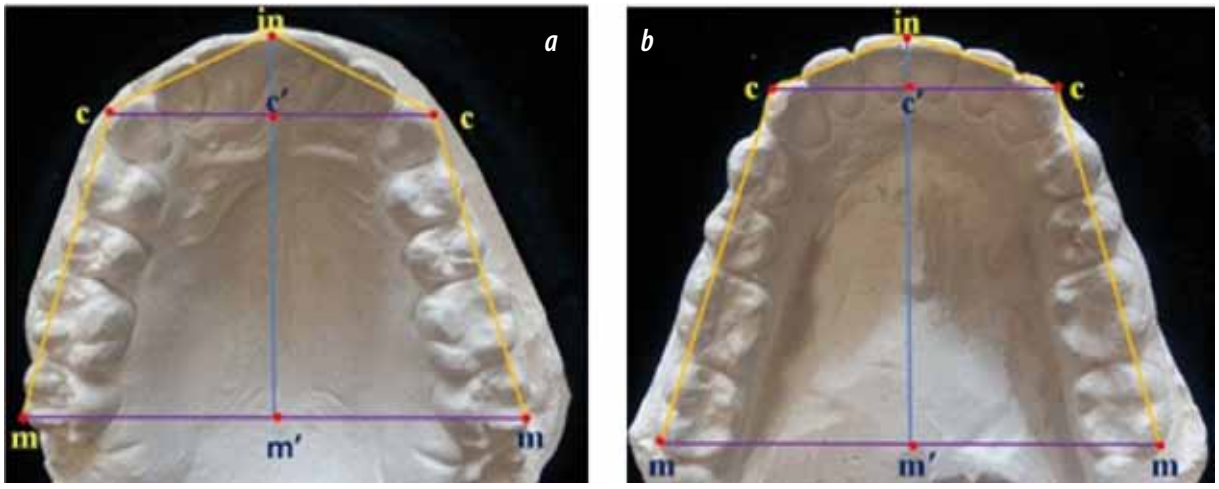



***Fig. 2.*** *Reference points for designing a dental pentagon on the upper (a), lower (b) jaw*

At the second stage, the arch graphic reproduction was done. From the *in* point, the point *o* was drawn down along the vertical line, for a value equal to the calculated radius, and then a circle was outlined, which, in case of physiological occlusion, passes through the canine points. The middle of the side lines (*c-m*) was marked as the *A* and *B* points. Further, perpendicular lines were drawn from points *A* and *B*, with an intercrossing on the *in-m'* vertical at a value equal to the length of the dental arch (the sum of crowns width of 14 teeth). These lines were marked as *A-D* and *B-C*, respectively. From points *C* and *D*, the distance to the canine points (*c*) or to the molar points (*m*) of the opposite side was measured, while the specified value served as the dental arch lateral segment radius (Fig. 3).

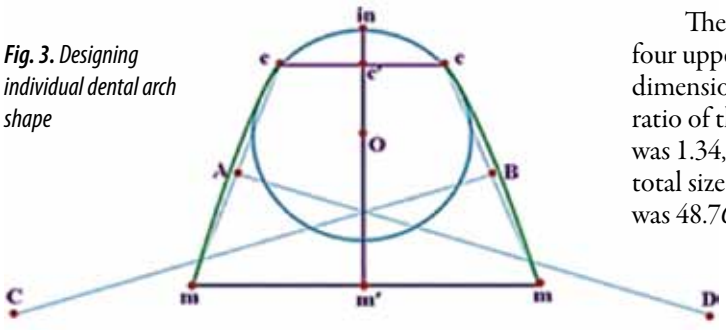
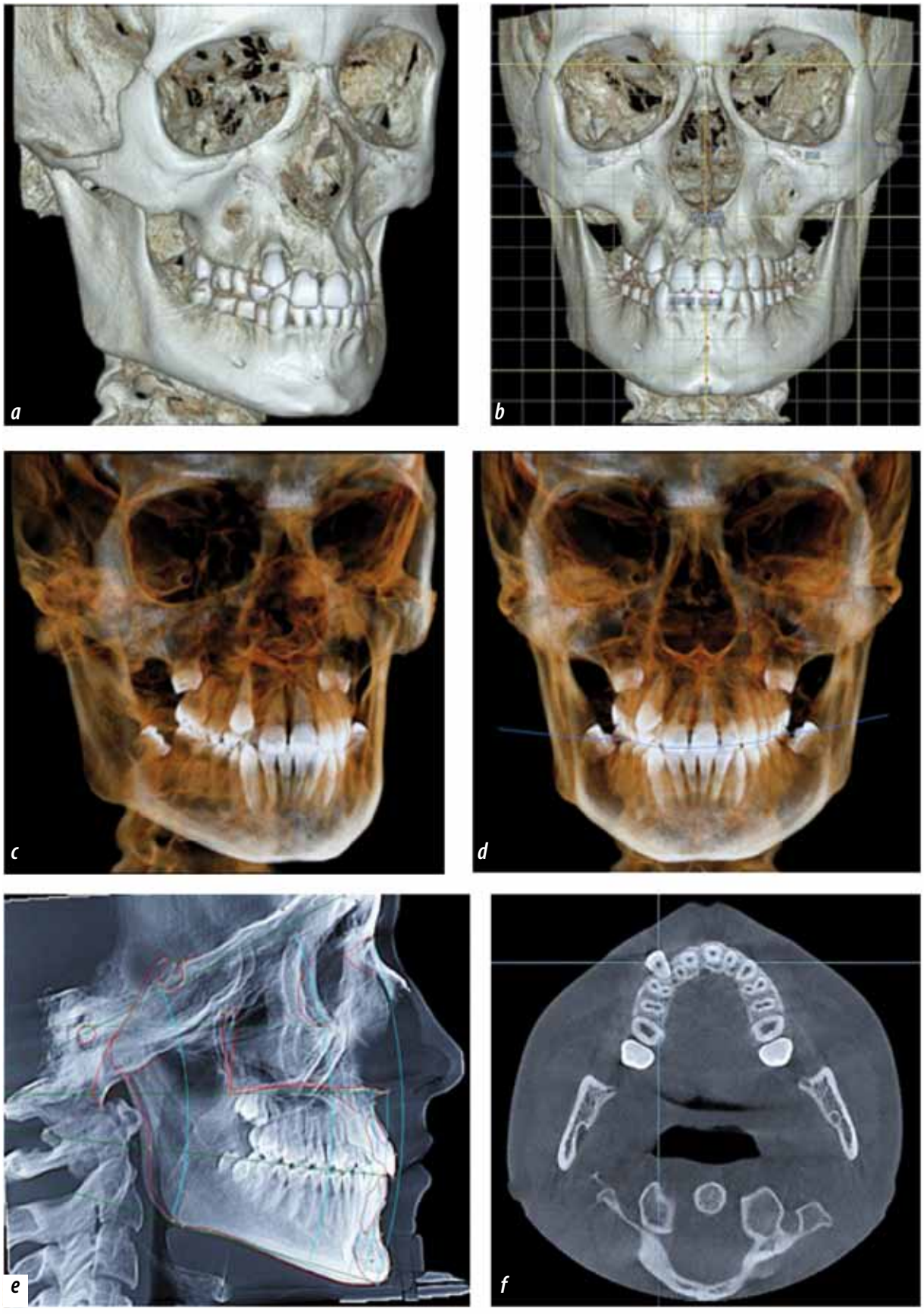



***Fig. 3.*** *Designing individual dental arch shape*

The effectiveness of the method for constructing the dental arch individual shape can be illustrated with a clinical example. Patient K., 18 y.o., came to the clinic complaining about the front teeth wrong location. A visual examination showed relative symmetry of the face; the vertical proportions were within the age norm. In profile, the upper lip does not reach the Riccets E-line. When examining the oral cavity, the Angel class I occlusion anomaly was diagnosed; the first molars were in a neutral position on both sides; the upper canine on the right was beyond the dental arch (on the vestibular side), and occupied a supraposition due to a lack of space in the dentition (Fig. 4).

Measuring the teeth revealed that the upper teeth dimensions correspond to the antagonists parameters, and belong to normodontia (Table 1).

The sum of the mesial-distal dimensions of the four upper incisors was 32.56 mm, while the similar dimensions of the antagonist teeth were 24.2 mm. The ratio of the upper vs. lower teeth size (Tonn index) was 1.34, which pointed at their proportionality. The total size of incisors and canines on the maxillary arch was 48.76 mm; on the mandibular arch — 37.66 mm,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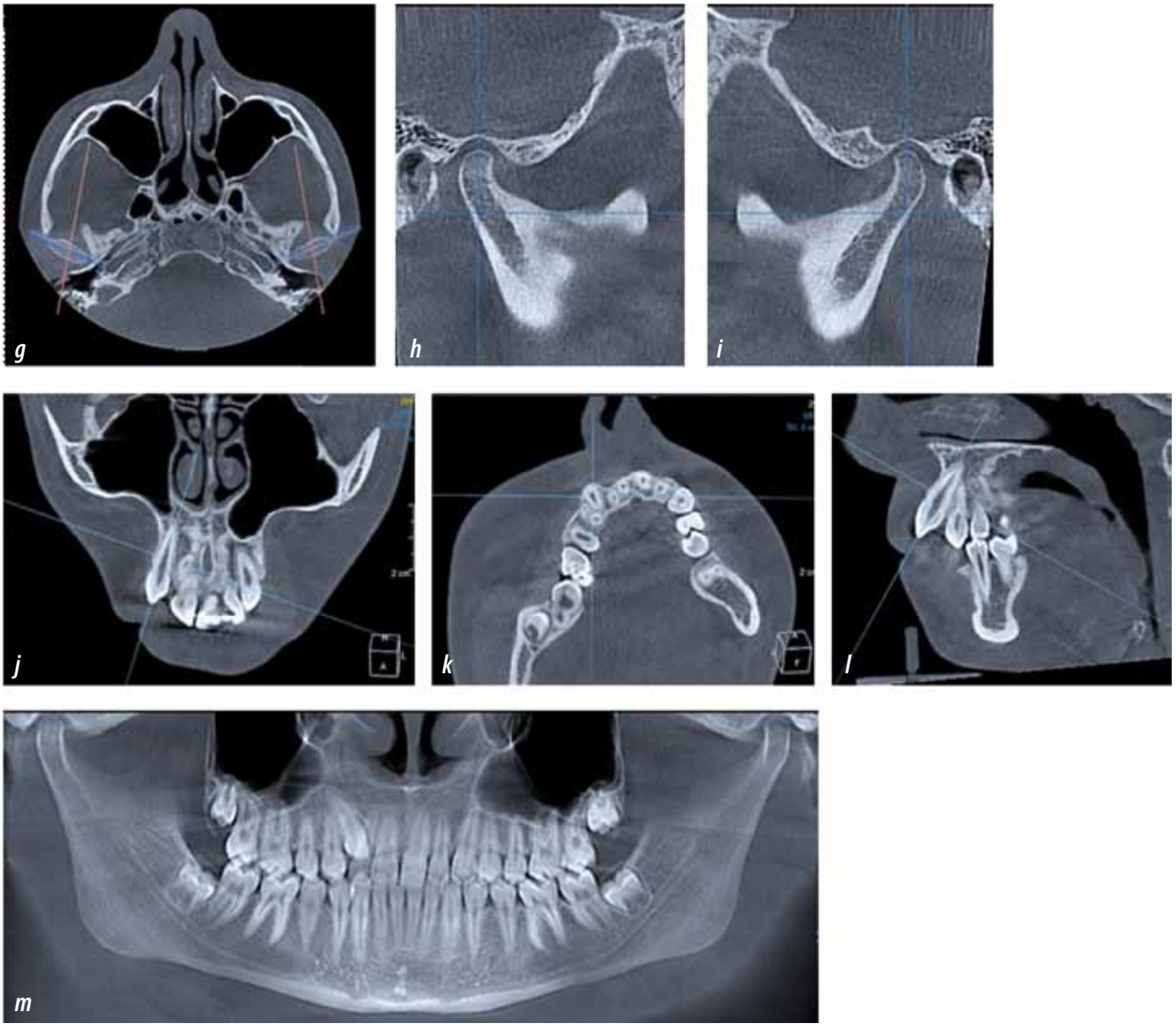


***Fig. 4.*** *3D visualization in the "Bone" option and VR mode in the sagittal (a) and frontal (b) planes; 3D visualization in the "Teeth" option in the sagittal (c) and in the "Teeth 2" option in the frontal (d) planes; Decoding a teleroentgenogram using the Sassuoni method (e); Maxillary axial reformat (f); Temporomandibular joint in axial (g), sagittal right (h) and sagittal left (i) projections; Topography of the canine in the frontal (j), axial (k) and sagittal (l) planes; MIP image of panoramic construction of the jaw bones (m)*

***Table 1.*** *Odontometry indicators, Patient K.*

| Examined dentitions | Dimensions of teeth that have a certain position in the dentition | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| Upper jaw | 9.04 | 7.24 | 8.1 | 7.4 | 6.72 | 10.28 | 9.75 |
| Lower jaw | 5.7 | 6.4 | 6.73 | 7.3 | 7.6 | 10.8 | 10.03 |

mandibular dental arch was 109.12 mm, this indicating a normodontia dental system.

Subject to the proposed algorithm, the width of the dental arches between the second molars (upper jaw — 58.6 mm, lower jaw — 53.21 mm) was measured. The value of the dental arch gnathic index (the length to width ratio) was as follows: on the upper jaw — 1.998 units, on the lower jaw — 2.051 units. The estimated parameters fall within the dolichognathic dental arch. A comparison of the gnathic and dental types allowed attributing the dental arches of both jaws to the protrusion type. In view of the types of arches and the proposed coefficients, the forecasted parameters of dental arches were calculated (Table 2).

***Table 2.*** *Forecasted parameters of dental arches (mm), Patient K.*

| Examined parameters | Dimensional parameter on dental arch | |
|---|---|---|
| | Upper | Lower |
| Front segment arch length | 40.66 | 30.93 |
| Front segment arch diagonal | 20.33 | 15.46 |
| Front segment arch depth | 10.16 | 6.49 |
| Front segment arch width | 35.21 | 28.07 |
| Circle radius | 20.33 | 18.41 |
| Dental arch diagonal | 55.22 | 50.52 |
| Dental arch depth | 46.80 | 42.94 |

The next stage implied the design of a dental diagnostic pentagon with a circle in order to evaluate the location of the front teeth as well as to compare the obtained graphic reproductions with the abnormal shape of the arch (Fig. 5).

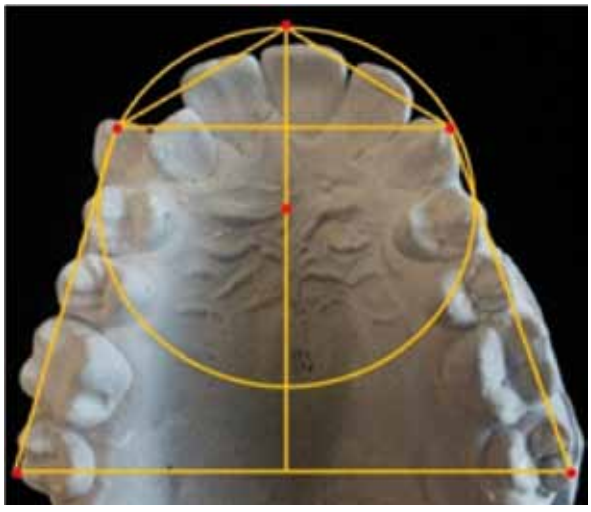

***Fig. 5.*** *Comparison of the upper jaw abnormal arch with the forecasted parameters*

The measuring of the parameters and comparing the abnormal arch with the graphic reproduction revealed a mismatch, which was most significant in the anterior segment. The diagonal of the anterior section on the right was 2.09 mm below the calculated values. The arch depth was shortened by 3.02 mm, while in the anterior part of the arch the mismatch was 3.11 mm. The inter-canine distance exceeded the calculated values by 1.84 mm, which was due to the vestibular position of the right canine. The diagonal of the right side of the arch was below the calculated values by 3.9 mm, and corresponded to the lack of space for the canine location, which determined the orthodontic treatment tactics using fixed equipment of mechanical effect. The sequence of changing the arches, braces installation on the lower jaw, and the retention period of treatment were performed following the Protocol for orthodontic patients. After the final stage of the treatment, the shape of the dental arches on both jaws matched the normal parameters and the calculated values as identified at the pathology diagnostics stage. The status of the occlusion, both in the lateral and anterior parts, matched the signs of physiological occlusion and the calculated type of dental arches (Fig. 6, 7).

Patient K's facial features basically revealed no change after the treatment of the dental arches abnormal shape. The smile features no buccal corridors, which makes the patient's face harmonious and aesthetic, as well as serves evidence to the treatment effectiveness (Fig. 8).

Given the above, employing the graphical research method at the stage of diagnostics and treatment working with patients featuring abnormal shape and size of dental arches, proves an effective tool, and is to be recommended for clinical orthodontics.

## CONCLUSIONS

1. A comprehensive approach based on an objective analysis of the quantitative (morphometric) and qualitative (clinical, visual) features of the dental complex allows obtaining a complete image of the normal or pathological status in terms of the dentition and occlusion, as well as taking timely preventive or therapeutic measures in view of the clinical indications.

2. Setting a diagnosis in orthodontics clinical practice is based on scientific knowledge, taking into account the age-bound norm and the patient's dental system development patterns, as well as the body's potential for self-regulation, on the one hand, and on the other — on the ability to interpret clinical signs and morphological parameters. Based on the outcomes of clinical and morphological conclusions, indications for preventive or therapeutic measures are developed, with forecasts made concerning the effectiveness of

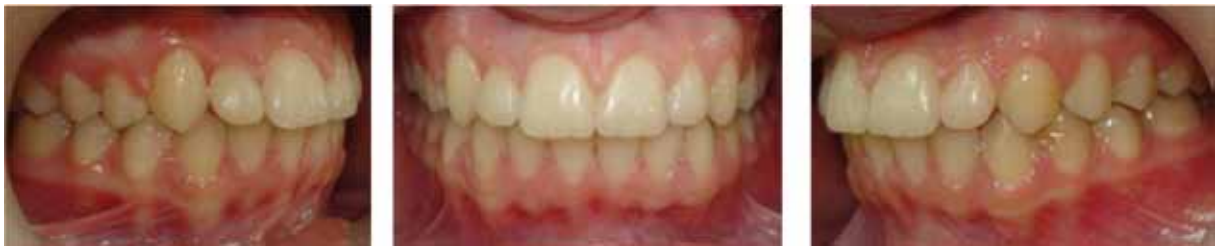

***Fig. 6.*** *Occlusal relationship of dental arches after orthodontic treatment, Patient K*

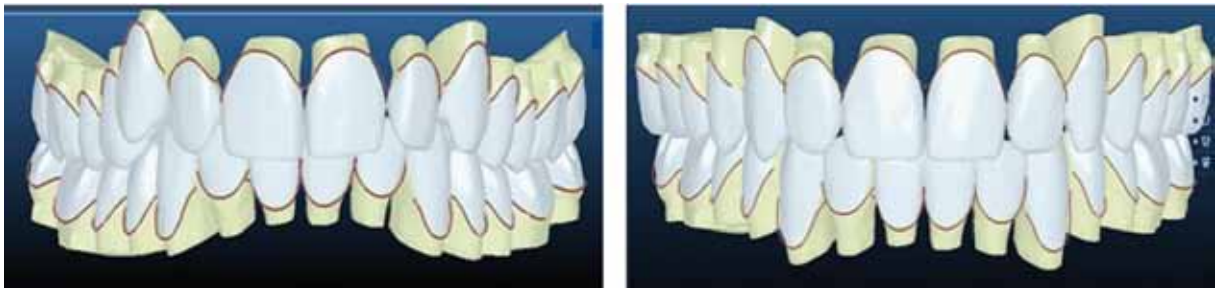

***Fig. 7.*** *Virtual diagnostic Set–Up model in the ORAPIX 3Txer 2.5.0 file (Japan), patient K; prior to, and after the orthodontic treatment*

***Fig. 8.*** *Facial signs, Patient K.; prior to, and after the orthodontic treatment*

treatment and the risk of developing the anomalies recurrence.

3. The effectiveness of therapeutic and diagnostic measures in patients with abnormal types of occlusal relationship has been proven through modifying the dental arches graphic reproduction method, taking into account individual dental features.

4. The first stage of graphic reproduction of the dental arch individual shape implies constructing a detailed pentagon whose base is the width of the dental

arch between the second molars, whereas the median sagittal line determines the dental arch depth. The upper sides of the pentagon (incisor-canine diagonals) run from the central inter-incisal point to the canine point, while the lower sides (canine-molar diagonals) connect the canine and the molar points. The second stage of graphic reproduction involves drawing a circle whose radius is to be calculated as the ratio of the sum of the square from half-width of the anterior part of the arch and the arch depth square to double-depth of the anterior part.

5. The study has proven clinical feasibility of the method to be employed for predicting the optimal individual dental arch shape through graphic reproduction in patients with Angel class I occlusion issues.

6. It has been illustrated that the effectiveness of therapeutic and diagnostic measures in patients with occlusion anomalies shall be achieved through following strictly to the sequence of dental arch graphic construction stages.

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