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# EDITORIAL



Dr. Georg Tyminski Editor-in-Chief

#### Dear colleagues,

Year 2021 is coming to an end. It has been a year of toughest hardships for many of us. We lost our loved ones, friends and colleagues. Especially hard this year has hit doctors and medical staff who led the fight against the covid-19 pandemic on the frontlines.

Our Editorial Board suffered a heavy loss. In October 2021 our friend and a rigorous scholar from Kazakhstan, pediatric surgeon Prof. Nurlan Akhparov died of COVID-19. Prof. Akhparov worked in medicine for 40 years and for 28 years he headed the Department of Pediatric Surgery at Scientific Center of Pediatrics and Pediatric Surgery, Almaty, Kazakhstan. Prof. Nurlan Akhparov performed over 10,000 surgeries and introduced 21 new surgical procedures in Kazakhstan.

However, our Journal gained certain achievements in 2021. It has been developing and we are proudly presenting 6 issues: five of them are already published and the 6th one is just on its way. The Editorial Board is becoming more international and interdisciplinary, which is positively influences the quality of the journal.

We wish all members of the editorial board, all our reviewers, authors and readers success and prosperity, sound health and happiness in New Year 2022! Let you in full swing enjoy the challenge of working in medicine. Let no boundaries curb your creative spirit either in your work or in your private life.

# TRIBUTE to PROF. NURLAN AKHPAROV

This time we honor Prof. Nurlan Akhparov - an outstanding pediatric surgeon from Almaty, Kazakhstan, our editorial board member, a reliable and enthusiastic friend, loving husband and father. Since September Nurlan fought covid-19 and no one could have foreseen this tragic end. On 9 October 2021 Nurlan Akhparov died aged only 61.

His credentials are impressive: MD, Professor, Academician of European Academy of Natural Sciences, Chief of the Department for Pediatric Surgery at Research Center for Pediatrics and Children Surgery, Almaty. Kazakhstan.

More than 40 years Nurlan Akhparov devoted to medicine. 28 years out of them he worked at Research Centre of Pediatrics and Pediatric Surgery. Prof. Akhparov performed over 10 000 surgeries and gave a second life to many children. In 1994 Nurlan Akhparov was one of the founders and all the time Chief of the Department for Pediatric Surgery until his last day. His research and practical specialties were thoracoabdominal surgery, coloproctology. Prof. Nurlan Akhparov was the first in Kazakhstan to introduce 21 new surgical procedures. Nurlan Akhparov filed 20 patent applications, 2 rationalization proposals, published 290 research papers among them 3 monographs and a manual for doctors. Prof. Akhparov supervised numerous MD theses and PhD dissertations.

Nurlan Akhparov received prestigious national awards: "Excellence in Healthcare of Republic of Kazakhstan" (2005). The prize «Ilkham» by Maecenas Club of Kazakhstan in the nomination «Science» (2007). The Republic Prize "Folk Hero" in the nomination "For Loyalty to the Profession" (2009). Prof. Akhparov was awarded with Robert Koch Medal (2016) and the Order of Alexander III (2017) for his outstanding contribution in medicine.

Prof. Akhparov served as an editorial board member of a number of journals: Archiv Euromedica, Pediatrics and Pediatric Surgery, Healthcare of Tajikistan and from 2018 - American Journal of Pediatrics.

For years we were privileged to welcome Nurlan at our medical events, to enjoy post-conference tours together... Our heartfelt condolences to the family and friends. Dear Doctor, we have so many memories, you will be forever in our hearts. In loving memory!

on behalf of Editorial Board of Journal ArchivEuromedica

**Georg Tyminski** Editor-in-Chief

# PROBLEMATIC ISSUES OF INFORMATION SUPPORT FOR MANAGEMENT DECISION-MAKING IN HEALTHCARE

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ABSTRACT — The purpose of the work is to analyze the main problem areas and the possibilities of introducing an information support system for making management decisions (controlling) in the activities of managers of modern medical organizations. The work used bibliographic, descriptive-analytical, general scientific, methods of system and content analysis. As a result of the study, the prospects for the use of controlling tools in preventive and clinical medicine, including in activities directly related to the treatment of patients, have been substantiated; a number of indicators have been proposed for monitoring and assessing the health status of the population; an assessment was made of the possibilities of using the decision-making information support system in the management of a medical organization; the main directions of further implementation of IT-technologies in healthcare practice are presented. The results obtained in the course of the study will significantly improve the quality of management decisions made and the effectiveness of the activities of modern medical organizations.

**KEYWORDS** — healthcare; information technology; controlling; clinical medicine, preventive medicine, management decisions.

#### INTRODUCTION

In a market economy, medical organizations are forced to participate in fierce competition for government orders, access to healthcare funds and the sector of paid services. Therefore, today the problem of introducing innovative technologies into the management system of organizations in order to increase the efficiency of their activities comes to the fore and becomes more and more urgent. Increasing the duration of a person's active life also requires significant investments in the development of the health care system, including in the training of highly qualified personnel, the introduction of innovative high-tech solutions based on the use of modern IT technologies in support

systems and in making clinical and management decisions. Despite the fact that health care expenditures are very heterogeneous around the world, it should be noted that the relationship between health care expenditures and public health indicators is not always direct. In this case, the key indicators are traditionally life expectancy, that is, the average age at death from all causes, and the infant mortality rate per 100,000 newborns, in other words, the number of children under 1 year of age per 100,000 births [9]. World Bank data are indicative and suggest that increases in health spending are not necessarily proportional to improvements in the quality of health care delivery. For example, when in the United States health care costs exceeded 8 thousand conventional units per person, and the average life expectancy approached 80 years, Japan, Portugal, Slovenia, Italy exceeded this bar at half the cost [1].

A significant contribution to this situation is made by the peculiarities of the geographical location of countries, mentality and a number of other reasons, however, the key factor, especially with life expectancy above the average level, is the efficiency of the health care system.

The COVID-19 pandemic and syndemia, the mutual course of a new coronavirus infection and a number of non-communicable diseases, such as diabetes mellitus, kidney and cardiovascular diseases, which worsen the prognosis and aggravate the course of diseases, have revealed a number of significant deficiencies in the health care system, one of which is the absence of a harmonious system of information support for making clinical and managerial decisions (controlling), which allows you to quickly receive timely and reliable information, both in individual medical organizations and at various levels of management of the health care system as a whole. Taking into account the limited funding for health care, as well as the increased burden on medical organizations in the context of the ongoing pandemic of the new coronavirus infection, the urgency of the problem of increasing the efficiency of the health care system, the effectiveness of management in health care is constantly increasing. One of the most promising ways to improve the efficiency of management is its information support, which makes the task of organizing a support system for making effective management decisions, wider implementation

of the controlling system and its corresponding tools in the activities of medical organizations, the so-called «medical controlling» [3, 4, 12].

#### **METHODS**

To achieve the goal set in the work, the following methods were used: bibliographic, descriptive-analytical, general scientific (analysis, synthesis, generalization), methods of system and content analysis.

#### **RESULTS AND DISCUSSION**

#### Controlling in clinical medicine

In the conditions of market relations and insurance medicine, controlling has serious prospects for implementation in healthcare practice, both at the level of management of structural and functional units and medical organizations in general (offices, departments, institutions, a network of institutions), and at the level of administrative-territorial units. At the same time, in various types of medical organizations, controlling tools can provide significant support in the work of both managers (heads of departments, chief physicians and their deputies, chief nurse) and attending physicians who are directly involved in the process of treating patients.

In each case, controlling will allow monitoring the indicators characterizing the patient's health (levels of temperature, saturation, blood pressure, heart rate, blood glucose, glycemic index, etc.). The monitoring and analysis system of these parameters can be considered as a controlling system, since it corresponds to its functions and tasks [5].

One of the main goals of the management of a medical organization — control over the expenditure of all types of resources — is successfully solved by controlling tools, while the powers of the head of a healthcare institution (chief doctors of hospitals, outpatient centers, etc.) economic independence, increasing the efficiency of controlling becomes more and more urgent [7].

Various medical and laboratory information systems (MIS and LIS, respectively), successfully introduced and actively used today in clinical practice [2], have become a specific tool for controlling in health care, allowing to streamline the distribution of the incoming flow of patients, eliminate queues for appointments to doctors, rationally plan the workload of laboratories , effectively use the existing funds of organizations, including beds, assess the quality of work of specific employees of the organization for the implementation of a differentiated system of incentives for employees, depending on the quality of their work. Controlling in the tasks of the healthcare organization

An equally important aspect of the application of controlling in health care is the use of its basic principles in the organization of the health care system of citizens.

As an IT tool at the level of the healthcare organization, it is necessary to bring clinical registers, which are from a technical point of view a medical information system, however, organized according to the principle of «all patients with a specific disease / condition in a certain territory» (region, country as a whole), as opposed to MIS, organized according to the principle «all patients of a particular health care institution with their diseases» [11].

We consider it expedient to single out today a number of indicators by which one can judge the state of health of the population in a particular territory. In the event that we ensure monitoring of the state of such indicators, which allow the relevant managers and governing bodies to make informed decisions, we can state the fact of the implementation of the controlling system in practice. In addition to the already mentioned life expectancy, maternal and infant mortality, it is advisable to include the following indicators among such indicators:

— The number of medical personnel (doctors, nurses) per population unit (a particularly important indicator of the overloading of the healthcare system, typical for work in the context of the Covid-19 pandemic);

Number of beds in day hospitals and in hospitals around the clock;

- Share of government spending on health care;

 Mortality from all causes in the context of age categories of the population (including by region the indicator allows you to find the most «problem» nosological forms);

— The incidence rate of the population, especially socially significant diseases;

 Tracking all processes occurring in medical organizations in real time and comparing target results with those achieved;

Postoperative complications and postoperative mortality;

— Maternal mortality rate (characterizes the quality of obstetric and gynecological medical care);

The prevalence of HIV infection;

— The proportion of infected pregnant women receiving antiretroviral drugs (an important indicator of both the prevalence of HIV and the likelihood of having a healthy baby from an HIV-positive mother);

— Morbidity and mortality from malignant neoplasms in the context of regions;

 The number of deaths caused by certain types of malignant neoplasms (allows to assess the quality of cancer care for the population).

In our opinion, important indicators at the population level are indicators of the «burden of disease» and «cost of disease».

«Burden of disease» is a group of indicators characterizing the economic damage from morbidity (6). The main indicators of the «burden of disease» are:

— years of life lost due to premature mortality (years of life lost, YLL: years not reached the value of life expectancy);

— years of life with disability (years lost due to disability, YLD — time of life lived with disability due to illness and injury);

— years of life adjusted for disability (disabilityadjusted life years, DALY: sum of YLL and YLD);

— healthy life expectancy (HALE: analogous to life expectancy minus DALYs).

At the moment, the international scientific partnership in the field of the global burden of disease, injury and risk factors, which includes the authors of this article, is monitoring the values of these indicators for different countries and territories (in particular, for a number of countries, the indicators are detailed to the level of administrative-territorial units the first level — the states of the United States, regions of England, regions of Russia, etc.).

As a subjective criterion for assessing one's own health, it is advisable to use the QALY (Qualityadjusted life-years) indicator, which characterizes the quality of life of patients and is assessed based on the use of a unified questionnaire. Due to the difficulties in collecting relevant information, a specialized study to assess the QALY in Russia was implemented only relatively recently [8].

The values of the listed synthetic indicators are important for the effective organization (reorganization) of the healthcare delivery system - these parameters, especially in the aspect of comparative assessment of the values of indicators in different regions, must be used by the executive authorities of different countries, as well as at the international level (on the scale of the World Health Organization).

If the indicators of the «burden of disease» demonstrate temporal characteristics, then the «cost of diseases» is characterized by financial indicators reflecting the monetary costs of treating a particular nosological form, including both the real costs of organizing medical care and lost profits in the economy as a result of disability and its reduction. However, despite the detailed assessment, such a study is extremely time consuming. A truly realizing in practice the controlling system today allows the transition from the global to the local burden of disease, when the decision-maker has not just aggregated data, but the values of the most important indicators, «tied» to real medical organizations or regions [10]. As the «resolving power» of the method grows, it becomes possible to take specific measures on a specific territory, which is relevant both at the local level and at the level of global organizations (for example, international organizations can plan targeted humanitarian missions) [13].

The analysis showed that at the moment in the Russian health care system as a whole, one can state the lack of specific controlling tools that take into account its specifics. At the same time, it seems logical that they should provide monitoring of indicators that are most consistent with indicators used (or potentially applicable) in the tasks of clinical medicine (as a criterion for the effectiveness of a particular product in a particular case) and healthcare organization (as a criterion for the effectiveness of a product in principle).

The introduction of individual elements of the controlling system into the activities of modern organizations already in itself increases the efficiency of management and facilitates their interaction at all levels, thereby contributing to survival in the turbulent world of market relations. However, it is possible to further increase the efficiency of their activities due to the transition from the use of individual elements to a full-fledged controlling system (a subsystem in the management system of a medical organization), which makes it possible to successfully implement innovative projects in the field of healthcare.

#### CONCLUSION

Thus, today all spheres of human activity, including the sphere of health protection, are significantly transformed under the influence of information technologies, widely using controlling tools, which not only simplifies communication, but also provides control over the state of indicators that allow predicting situations, taking optimal and timely management and clinical decisions.

In the conditions of a market economy, the emergence of economic independence for the subjects, the growth of competition, the need to increase the efficiency of management increases, which is impossible in the absence of reliable information support.

The development and implementation of an automated monitoring system for the indicators proposed by the authors, their use in the tasks of increasing the efficiency of management of medical organizations and the health care system as a whole, can be considered elements of controlling. In view of the digital transformation of all spheres of activity, it is already possible to state the presence of elements of the controlling system in management practice, for example, IT solutions for personnel accounting, the movement of financial flows and material resources. Such solutions not only allow improving the efficiency of management and interaction, today they have become an integral part of professional human activity. A qualitatively new result will be achieved both by the integration of individual elements into a full-fledged controlling system of an economic entity (start-up, organization, medical institution), and by the creation of a unified controlling system for the health care sector as a whole.

Providing information and analytical support for the management process, controlling provides significant assistance to the head of a modern medical organization in the process of implementing all types of planning of its activities, coordinating the work of structural and functional units, analyzing the main indicators of the institution as a whole and its individual units, assessing the availability and quality of services provided to patients medical care.

Thus, controlling today should be focused on the continuous improvement of a unified system for managing medical activities in terms of coordinating the development and achievement of the set goals. It should ensure, at all stages of the management cycle, the integration and coordination of the functioning of systems and processes based on standard procedures. When organizing and building controlling, it is necessary to strive to ensure that its information base is provided by a unified information system that works according to general rules for all subjects of the healthcare management system.

The authors declare that there is no conflict interests and any third-party financing.

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# ANXIETY AND FRUSTRATION DURING THE COVID-19 PANDEMIC

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ABSTRACT — The lockdown and quarantine period set by authorities around the world in order to prevent the spread of the SARS-COV-2 virus has had a significant impact on the mental health of people around the world. The present research, carried out by qualitative methods, aimed at identifying the sources, but also the ways of expressing anxiety, frustration and anguish due to the Covid-19 pandemic and the measures to prevent the spread of the virus. The research was carried out on subjects of Romanian nationality, especially from the North Eastern Region of Romania. The main results of the research are: an extension of the medicalization of social life, the awareness of one's own finitude and the experience of helplessness, as sources of anxiety and frustration, and an accentuated social response to the risk society, manifested as a revolt against the authorities and the need to humanize every day life.

**KEYWORDS** — pandemic, anguish, frustration, medicalization of social life.

#### INTRODUCTION

Anxiety is defined as a pathological state of restlessness, of undetermined fear, which is often accompanied by physiological disorders. Frustration is a consequence of the "deprivation of one's right", also understood as harm, deprivation of satisfaction, deception, disappointment, the condition of an individual who, due to an obstacle or inhibition, cannot fulfill a wish or satisfy an enjoyment ("The New Explanatory", 2002).

The research that is the subject of this study was conducted based on an interview applied to a number of 103 people and was aimed to highlighting the phenomena of anxiety and distress due to the restrictions imposed during the pandemic.

#### THE CONTEXT OF THE RESEARCH

The Covid-19 pandemic causes the whole of society to be restricted, with or without real grounds for public health protection, restrictions imposed on the freedom of movement, on specific social behaviors such as social distancing, wearing a mask, transferring most of the public activity to the online environment (virtualization of the social space) and so on.

#### THEORETICAL APPROACH

A series of studies (Damian et al, 2020; Dogar et al., 2020; Huidu, 2020; Hunea et al., 2020; Mishra, 2020; Oleshko et al., 2020) conducted during the first wave of the Coronavirus pandemic, during which almost all countries in the world were in a state of lockdown and quarantine, show that the spread of the SARS-COV-2 virus has a significant impact on the population in various communities. According to Salari et al. (2020), during the pandemic, psychological measures must be taken to improve the mental health of the population, especially of those population groups considered to be vulnerable. As the effects of the pandemic, especially on mental health, are still manifesting (Loue& Lamb, 2020), the results of a research on the anxiety and frustration felt during the pandemic by the population may be beneficial both therapeutically and pragmatically, for people involved in the construction of public health policies (Jaradat&Stupar, 2020), as well as for clinical psychologists and those performing psychotherapy and psychological counseling for the benefit of people who have suffered as a consequence of the pandemic.

#### Anxiety — operational definitions and etiology

Anxiety is distinguished from fear ("Anxiety", 2021) by the vague nature of the sensation that appears in response to an unspoken danger (sometimes only intuited), while the second state appears in response to a clear and obvious danger. Anxiety is a subjective response to an internal emotional conflict, the causes of which may not be obvious to the person experiencing the state of anxiety. When the state of anxiety is intense — recurrent or chronic, and cannot be justified by the fact that it would be a response to stressful stimuli in everyday life, it is considered to be an emotional disorder.

Anxiety Disorder is one of the most common pathologies (Bystritsky et al., 2013) that affects the mental health of the population worldwide. Kessler et al. show that this type of disorder affected 13.3% of the U.S. population in 2013, and the percentage was higher in 2013 compared to previous years. Anxiety disorders is the most important sub-group of mental illnesses that occur worldwide, but are being studied mainly in developed countries.

The subgroup of mental illness represented by anxiety disorders includes a number of syndromes, of which DSM-5 (The Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition) focuses on the Generalized Anxiety Disorder (Reynolds & Kamphaus, 2013). This disorder is caused by excessive anxiety and worry that lasts for at least 6 months. In DSM-4, anxiety disorders included: Panic Disorder with or without agoraphobia, social phobia, specific phobias about animals, the environment, injections or various situations, Post-Traumatic Stress Disorder, Acute Stress Disorder, Obsessive-Compulsive Disorder, and other anxiety disorders. DSM-5 redefines anxiety disorders, eliminating post-traumatic stress syndrome and obsessive-compulsive disorder, which it redefines as separate classes of mental disorders (Bystritsky et al., 2013).

Prior to the advent of DSM-5, which builds a whole class of anxiety disorders, Social Anxiety Disorder was known as Social Phobia, but it was reclassified as an independent disorder (Heimberg et al., 2014).

Social anxiety can manifest itself as a withdrawal from the social environment and isolation, often accompanied by depression and body dysmorphism (BDD — Body Dysmorphic Disorder).

Cultural differences can be found in the rationalization of fear — a key element in diagnosing anxiety disorders, differences that must be taken into account when talking about social anxiety. We mention that DSM-5 introduces the concept of Cultural Syndrome, with significant influences in terms of anxiety.

Despite the different symptoms of the various forms of social anxiety generated by the cultural context, the common element is the gap between the social expectations perceived by the individual and the perception of one's self, according to which the individual considers themselves as not being able to meet social expectations or to perform their social role, in accordance with the constructs of the society to which they belong. Thus, social anxiety creates a disorder that puts the individual in a position of suffering — acute or chronic — and performative inefficiency of the tasks or roles they have to perform.

### METHODOLOGY

#### Research aim and objective

The research aimed to highlight the phenomena of anxiety and distress due to the restrictions imposed during the pandemic.

The objective of the research is to highlight and analyze the possible manifestations and risks created by anxiety and frustration during the pandemic.

The assumption on which the research is based is that the Coronavirus pandemic has negatively influ-

enced the mental health of the population, by inducing anxiety and anguish among a significant part of the population.

#### Sampling and data gathering

This research on anxiety and anguish during the Covid-19 pandemic took place in April-June 2020 — the period when the countries of residence of the research participants were in lockdown, because the authorities imposed strict measures to quarantine some localities or regions and general measures of restrictions on traffic and travel, isolation and physical/social distancing, the obligation to wear a mask, the interruption of economic activities that were considered non-essential as well as the interruption of educational programmes.

The sample included 103 persons of Romanian nationality, of which 70 are residents of Romania, 12 are residents of the Republic of Moldova and 6 are residents of Austria, Spain, Italy, Belgium, Germany, Great Britain. Another 15 persons did not declare their country of residence. The structure of the sample by gender was: 62 female, 31 male and a number of 7 people did not specify their gender. The age of the interviewed persons was between 18-24 years (47), 25–30 years (26), 35–45 years (14), 45–60 years (9), 60-90 years (3). ), unspecified (4). Regarding the environment of origin, 42 interviewees come from the rural environment, 25 from the urban environment, and 36 did not specify their residence. Regarding the level of education, research participants stated they had graduated from higher education (63) and secondary education (31), while 9 persons did not declare their status as regards formal education.

The research was conducted with the help of unstructured interviews, applied online or by phone.

The analysis of the results was performed using the Grounded Theory method. In this paper, we partially present the results that were included in the category Anxiety and anguish. The results included in other discursive categories will be the subject of future articles.

### **RESULTS OF THE RESEARCH**

Contrary to our initial assumptions that the occurrence of anxiety and anguish during the pandemic should be very common and that various explicit symptoms of these conditions might be detected possible anxiety disorders, there have been a number of responses of some participants in the research that they were not severely affected by the pandemic, although they had distressing experiences during the pandemic, but did not identify them as disorders or suffering, considering them changes in mental mood, which do not affect self-expression socially (T.E.).

#### Measures imposed by the authorities to prevent infection a source of anxiety and frustration

A source of stress are the measures taken by the authorities to prevent the spread of the disease themselves, which the subject respects, because they understand the need for such measures, while being aware of the mental and emotional burden they bring to citizens, both by enhancing the awareness of existing risks, as well as the limitations to which citizens are subject: *I can say that I want to respect these rules from now on, but I do not know how I will resist mentally* (B.A.).

#### Dissemination of apocalyptic scenarios

One element that led to increased anxiety and even panic among the population was the *spread of apocalyptic scenarios*(A.J.), which included an exaggerated number of deaths, an extreme contagion of the virus, almost impossible to counter and avoid. The media insisted on presenting *fatal images* (A.J.), including *extreme visual violence* — whole rooms full of black bags, containing the bodies of those killed by Covid-19 in Italy, Spain etc. These images could be associated in the collective mind with similar images from movies with post-*apocalyptic scenarios*, in which a pandemic reduces the planet's population to near complete annihilation.

On the other hand, there were interviewees who stated that they had personally experienced the infection with the virus, in a more or less severe form. Among them, there are people who believe that the severity of *the symptoms of the virus is exaggerated*, compared to their *own experience of the disease*. However, there were also people who *needed hospitalization* either themselves, or members of their family or people from their group of acquaintances/relatives, or who even had deaths among acquaintances.

#### Conditions of hospitalization as a source of anxiety

There have been people who said that a source of anxiety — sometimes considered the most intense is related to the *conditions of hospitalization* (A.M.C.) and the lack of medication and medical supplies, but also poor care and even the inability of the patient infected *with Covid-19, who is hospitalized to communicate* with their family. A high degree of anxiety was shown by people at risk in terms of the severe forms of the Covid-19 disease, whether it is the elderly or those suffering from comorbidities. The highest imbalance appeared on the mental side. [...] The stress was much higher than in a healthy person, because the disease itself is of an affective-emotional nature and I knew for sure that if I contracted this virus, the consequences could be much more drastic and, for example, that sometimes, there were imbalances in my blood sugar and in my physical and

#### *mental condition (G.G. — type I diabetic person, dependent on insulin).*

Similarly, another person states that one of the sources of anxiety is the possibility of contracting a disease other than Covid-19, which would require *contact with the hospital environment* and, due to improper conditions in Romanian hospitals, lead to a Covid-19 infection and the development of a serious form of this disease. The subject even considers that *the highest risk of infection is in the hospital environment*, for patients with other diseases or for healthcare professionals

# Fear of infection related to the communication of the subjective experience of the disease

The interviewees generally stated that the *fear of infection* arose when they found out that people close to them had contracted the virus and that it manifests itself in quite / or even very serious forms. Relating to others has induced forms of fear, anguish or even anxiety. In some situations, fear is mentioned as being explicitly manifested in relation to the most vulnerable people in their own family (A.A.), (D.A.B.) or among those close to them. A *stoic attitude* is signaled even in *the elderly*, who accept with some serenity the prospect of a possible illness and even the loss of their own lives, but show feelings of fear for the life and health of those close to them: *The suffering of others hurts more than my own suffering* (D.S.).

#### Anguish and self experiencing

Respondent T.E. shows that the lockdown changed his way of life, causing him to experience anxiety precisely because of these changes, but which he subjectively does not recognize as a direct impairment of his mental health, because his self-expression was not affected, nor the perception of his own self, but only his quality of a moral or social agent.

As a "synthesis" of the states of anxiety and frustration that one of the interviewees felt, she states that: *I somehow felt that my life was gone, this truth brought disappointments, frustrations* (I.E.). *The feeling of losing one's life* — in fact, its essence — correlated with the lack of freedom of movement and the depreciation of communication, that placed the person in a *situation of non-self*.

#### The emotional availability of the subjects

There were subjects who showed that their *avail-ability* and *emotional state* changed during the lockdown state, as they went through periods of intense emotional arousal, followed by periods of understanding and acceptance of the situation. Such fluctuations were apparently determined by the type of information to which the subject had access and the way such information was interpreted, but also by the ability to find alternate sources of activity, which require as little travel as possible outside their own home and limiting the physical interactions with other people: *I noticed several changes in my emotional condition throughout this period of time. In the first week or so, let's say, in the first two weeks, I was very motivated, inspired. In week three, four I was very depressed, bored, a period in which I thought a lot about life, about the meaning of life* (C.I.).

#### Condemnation to suffering as an expression of the frustration felt by the population

One of the interviewees showed that she felt condemned by this pandemic: *I felt that I would be condemned and that this is a disaster for all people* (N.N.3). *The feeling of being condemned to suffering* was reinforced by the abundance of news in the media about the pandemic and their annoyance, as well as the panic-like behavior of a large part of the population. The obligation to stay at home and the transformation of this need into an obligation — and, at the same time, into an official public policy — strengthened the interviewee's conviction that the crisis situation will be a long-term one: *it will last forever and we will be forced more and more to stay at home* (N.N.3).

The lack of perspectives regarding the return to normalcy is seen as an incarceration in one's own home, without being guilty, and this is perceived in the case of the person mentioned above as a personal and collective misfortune, at the same time.

# Fear of death and the confrontation with the experience of one's own finitude

The approach to the pandemic has made many face their fear of death, and of course there have been people who have even prepared for imminent death. A simple fever automatically leads, under the effect of panic, to the thought of infection, and this generates anxiety, by anticipating one's own death, but also the effects it will have on those close to them. People who, during the pandemic, exercise *tasks that involve direct contact with the public*, experience an additional anxiety, related to the fact that they could be the source of *infection for their customers* and, implicitly, the cause of their possible death.

Symptoms of pandemic-induced anxiety disorders

*Fear for one's own health* is also present in the minds of the interviewees (D.A.B.), (D.M.C.). Stress caused by *prolonged isolation* (for two months) also caused other health problems — related to, but not necessarily caused by Covid-19 (D.A.B.). Symptoms associated with stress and anxiety include *fatigue*, *increased nervousness and irritability: I am always tired*,

although I do not do much, I also get angry very quickly from nothing. I get angry quickly and I can't concentrate on absolutely anything, I can't rest properly and I get very stressed (S.R.).

#### Coping mechanisms

In the case of some interviewees, the *first news* about the spread of the virus and the outbreak of the pandemic caused *panic*, later they declared that they adopted a coping strategy — in the sense of accepting the disease as *an inevitable reality*, that will accompany us in the long run, even after the end of the lockdown period (NN1). Thus, a reason for anxiety is the *inability to predict the duration of the pandemic* — both generally, until the return to a *normal post-pandemic life*, and in particularly, *during the lockdown period* which, once prolonged, can have consequences in terms of concerns regarding the *budgetary resources* of the person and their family, their state of health etc. (A.O.).

#### The paradoxical approach to stress as a way of coping

Situations were presented of elderly people who, despite the ban on leaving home, were caught taking walks or having trips that they did not make before the emergency state was declared. Many did not comply with this measure. At least in my neighborhood, I see dozens of elderly people, who didn't go out before the pandemic, walking around every day (B.R.). We attribute this attitude to the development, by the respective persons, of coping mechanisms for the pandemic situation, in which the feeling of freedom is more important than the feeling of security. The ban on leaving home was imposed to the entire population of the country, not only to those in quarantined localities. The exceptions to this rule were attending to current needs, making supplies, traveling to work, taking care of sick people etc., and could only be done on the basis of the declaration on one's own responsibility regarding the destination, the route and the duration of the journey. For the elderly, time slots have been set, in which there was an almost complete restriction on travel.

#### From the awareness of the risk associated with the pandemic to the risk society

Awareness of the severity of the virus has generated anxiety and panic, which we attribute to risk awareness (A.A.) for one's own health and the *emergence of radical lifestyle changes*. The two components lead us to the idea of the awareness of the fact that people live in an increasingly deeply medicalized risk society. *The virus is considered to be much stronger than we choose to perceive it* (A.A.). We note that the perceived severity of the public health problem raised by Covid-19 infection is considered a choice and that the interviewee (A.A.) considers that, for various reasons specific to each person, the individual chooses to perceive the virus less severely than it is in reality, despite messages from the media and warnings from national and international authorities.

Interviewees express concern about the severity of the current pandemic, that may affect their future — not just medically (D.A.B). Some even appreciate that mass immunization is a solution to eliminate or diminish the long-term effects of the pandemic (L.B). Interviewees expressed concern about the possible deterioration of the economic situation and the emergence of a crisis (A.B.).

#### Legitimacy and revolt in the fight against the pandemic

Another feeling about the situation caused by the pandemic, described by the interviewees, is that of *revolt: I felt the need to protect my family and I knew I could not do it alone, and that revolted me* (S.P.). A special mention made by those who stated that they felt feelings of revolt is related to restricting and / or limiting access to medical services, other than those intended for the care of people infected with Covid-19: *I was outraged that we were not allowed to go at hospitals for a routine consultation or for medical tests* (MT).

#### *From inefficient public communication to infodemic*

Another major cause of anxiety among the population is the *lack of communication* (A.B.), either between citizens — aggravated by *inefficient public communication*, or because of the *large number of false news*, which makes public communication less credible and / or misperceived by the population.

There were also opinions that exaggerate the severity of the infection, people who had mild symptoms, that would not normally require the intervention of a doctor, who turned to the emergency medical services who panicked and accentuated the panic of those close to them, on the one hand, and endangered the health system's ability to respond to truly emergency situations, on the other hand (D.B.).

# *The graduality of anxiety feelings and the panic epidemic — sources of degradation of the mental health of the population*

Those who express their anguish rank it from minor states of fear regarding the pandemic phenomenon, to real states of "*horror*" to the danger posed by the virus, especially in the context of other systemic crises.

In addition to the Coronavirus pandemic, the origin, size and effects of which were partially unknown and remained relatively little known or at least controversial at the time of this study, the population faced an *undeclared panic epidemic*, which affected *the mental*  *health of the population* to an extent equal to or even greater than the effects of the Covid-19 infection itself, except in severe and very severe cases. Although it was not declared aa a pandemic, because the transmission of panic is not documented as a community phenomenon, *the general state of anxiety and panic of the population* had effects that have not yet been highlighted and measured, but which, in our opinion, *accentuated the medicalization of society* and *the dependence on the therapeutic act* — whether we are talking about a medical, psychological, social or spiritual attendance.

#### CONCLUSION

Among the most important results obtained from the analysis of the interviews, we list: awareness of the increasing medicalization of society, awareness of today's society as a "risk society", distrust of the authorities' ability to manage the pandemic, distrust of the real dimensions of the pandemic and an easier acceptance of fake news on the pandemic, on the ambivalent attitude of the authorities, the refusal of hospitalization — even in the context of Covid-19 infection, justified by the perception of the inability of the Romanian medical system to manage the crisis, as well as the risk of authoritarian behaviors, interpreted as "white dictatorship".

The constituent elements of the anxiety generated by the pandemic are: the social construction of specific forms of anxiety, generated by the perception of disease risks, loss of quality of life, diminished social, personal and economic security, fear of the other — as a possible vector of infection, distancing — not only physically, but also socially, in the sense of degrading interpersonal communication and public trust, not only in the health system or in public institutions, but also in each other.

Our research also highlighted the distrust in medical institutions and public health systems, which is widespread among the population and strengthened by the inefficient communication of the importance of the measures to prevent the infection with Covid-19 and the false news (the infodemic) that health systems and authorities generally failed to combat.

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# DEPRESSION AND ATTITUDES TO DEPRESSION AMONG STUDENTS OF A RUSSIAN MEDICAL UNIVERSITY DURING THE PANDEMIC CORONAVIRUS

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**ABSTRACT** — The paper analyzes the attitude towards depression and the level of depression among medical students. Astrakhan State Medical University (Astrakhan, Russia) conducted a survey of students on their attitude towards depression, whether they consider depression a dangerous disease and whether they believe that it is necessary to consult a specialist in the presence of depression. The data of the depression level research and coping strategies among medical students are presented. In the course of study, it was found out that students know the symptoms of depression and the majority of them assess the effect of depression on their health correctly, however, they are not sufficiently informed about the treatment of depression and are biased against antidepressants. More symptoms of depression were diagnosed among junior students than that among senior ones. We identified that the students cannot adequately assess their mental condition and determine whether they have depression or not. It should be noted that students are practically not informed about such a disease as depression and it is necessary to carry out psychoeducational work with student youth.

**KEYWORDS** — attitude towards depression; medical students; coping strategies; depression; questioning; seeking social support; psychological help.

#### RELEVANCE

Depression is a serious emotional disorder that affects people of all ages, including children and adolescents [5]. It is characterized by a steady state of sad and irritable mood and loss of interest and pleasure in life [4]. These feelings are accompanied by a number of additional symptoms that affect appetite and sleep, the level of activity and concentration, as well as wellbeing [6, 7]. Depression is one of the most common mental disorders. WHO estimates that it affects more than 300 million people of all age groups. In adolescence, the overall prevalence of depression is 15 to 40%. In extreme cases, depression can lead to suicide,

one of the leading causes of death among young people aged 15-29. According to WHO forecasts, depression may come out on top in the world, overtaking today's leaders - infectious and cardiovascular diseases. Endogenous and exogenous depression are distinguished, endogenous depression is considered the most severe, in which it is necessary to consult a specialist. The effective treatment for depression is medication and psychotherapy [1]. Student youth falls into the risk group for developing depression and is considered to be the target group which is necessary to carry out preventive work for the early active detection of this disease with [8]. In general, it can be argued that depressive states significantly reduce a person's quality of life, vitality and interest in life.In the study of L.V. Kochorova, V.S. Skripova, A.S. Slivka. [2] it was found out that the majority of the surveyed students had symptoms of anxiety and depression, and a third of the surveyed students were ready to receive psychotherapeutic assistance. In the work of A.A. Yezhov, A.A. Parsadanyan. [3] the symptoms of severe depression and depression of moderate severity among studentsare characterized by the phenomenon that most of the time they are occupied only with themselves and their own thoughts and problems. During this difficult period, the personality is undergoing a restructuring of the psycho-emotional sphere, adaptation to new conditions, and the development of new coping strategies. Coping strategies are behavioral strategies that are characterized by flexibility and purposefulness. The study of the level of depression and attitudes towards depression in a sample of medical university students seems to be especially important in connection with the high academic load, longer training in comparison with students of other specialties. An additional risk factor is the epidemiological situation associated with coronavirus infection. Thus, the problem of studying the emergence and spread of depressive disorders at a young age is relevant today. Hence, there is an urgent need to study attitude towards depression in students and to determine the level of depression in the student environment. This topic is also relevant in connection with the spread of depressive disorders, the ongoing coronavirus pandemic and the creation of preventive

measures in the early stages to normalize the psychoemotional state of students, and increase awareness of depressive disorders. The results of the study will help to contribute to the field of differential diagnosis of depression among students, as well as to the prevention of depression and the reduction of negative consequences.

#### The aim of the study

is to study the attitude towards depression, identify the level of depressive symptoms and coping strategies in the student environment.

### MATERIALS AND RESEARCH METHODS

A survey on the attitude towards depression and psychodiagnostic examination of students was conducted in the Astrakhan State Medical University, at the Department of Psychology and Pedagogy. The research was done in December 2020. The comprehensive study involved 80 students of a medical university in the number of 1-5 courses, studying in the specialties "General Medicine", "Pediatrics". The average age is 20.2 years. The following methods were used in the study: 1) the author's questionnaire "Attitude of medical university students to depression", which allows to determine the attitude towards depression, whether they consider depression a dangerous disease, whether depression affects human health and whether they believe that it is necessary to turn to specialists when the presence of depression; 2) the level of depression was diagnosed using the scale (test questionnaire) A.T. Beck (Beck Depression Inventory), adapted by N.V. Tarabrina, which includes 21 categories of symptoms and complaints [9]. Beck's test questionnaire includes 21 statement questions that concern the behavior, thoughts and feelings of the test- taker in the past 2 weeks; 3) the methodology "indicator coping strategies" by J. Amirkhan (adapted by N. A. Sirota and V. M. Yaltonsky), designed to diagnose the dominant coping strategies of the individual [10]. Further processing of the data was carried out using the Student's t-test to identify the significance of differences, the K. Spearman's rank correlation coefficient for the correlation analysis of the data obtained. The SPSS-22.0 software package was used. During the study, a positive attitude to the situation and interest in the expected results were noted; there were no refusals during the survey. The research results are presented in the figures.

#### **RESULTS AND DISCUSSION**

The results of the questionnaire survey "Attitude of medical university students to depression" showed that 67.5% (54 people) experienced symptoms of depression in themselves or their loved ones at different periods of time. For example, 40.0% of them (32 interviewed students) noted that they themselves experienced a state of mild depression, significant stable and low mood and loss of appetite, 17.5% (14 people) observed a depressive state in their parents for various factors, such as stress or psychosomatic illnesses, and about 10.0% (8 interviewed respondents) experienced depressive symptoms in their friends and close social environment. 32.5% (26 students) did not experience a state of depression. In the course of the survey, students of the medical university were asked to assess the severity and danger of depression as a disease. As a result, it turned out that 37.5% (30 people) do not consider depression a dangerous disease that needs to be taken seriously. The average score for assessing the severity of depression in comparison with other diseases is 6 points on a ten-point scale among the studied students. This result is an alarm.

The questionnaire "Attitude of medical university students to depression" included questions that reveal the awareness of medical university students how depression affects the quality of life and health of people. It was found that 55.0% (44 interviewed respondents) noted that in a state of depression, a person experiences apathy, loses motivation for various types of activities (training, work, hobbies, etc.); 40.0% (32 people) indicated that in a state of depression suicidal thoughts can occur in a person. About 5.0% (4 interviewed respondents) associate the presence of a depressive state with increased conflict, irritability, emotional stress, as well as the manifestation of aggressive behavior in people. In the studied sample of medical students, opinions were divided regarding the effect of depression on a person's somatic health. The influence of a depressive state on health and on the quality of human life is noted by 75.0% (60 people). Depression only in some cases can affect the psychosomatic state of a person is noted by 10.0% (8 surveyed students). The absence of a connection between a depressive state and a healthy state was indicated by 15.0% (12 surveyed students).

The next block of questions in our questionnaire concerned what helps to cope with the state of depression and its symptoms. 47.5% (38 respondents) answered that a person in a state of depression should "pull himself together" and direct his activity to improve his mood. 30% (24 respondents) noted that in a depressive state and in depression, it is necessary to seek help from a specialist (psychologist, psychiatrist or psychotherapist). As an effective remedy for depression and anxiety, 15% (12 students) offer good rest. And 7.5% (6 people) noted that doing important things helps to cope with depression and neuroses. For help in a state of depression, 42.5% (34 respondents) consider it necessary to consult a psychologist, 42.5% (34 respondents) — to a psychotherapist, 5.0% (4 respondents) — to a psychiatrist and 10.0% (8 respondents) do not consider it necessary to consult a specialist. This result is cause for concern. Treatment of depression without medication is preferred by 85.0% (68 students), students are afraid of the consequences of using antidepressants and believe that addiction to them is possible.The second stage of the study was to determine, using psychodiagnostic testing on a scale (test questionnaire) A.T. Beck (Beck Depression Inventory), adapted by N.V. Tarabrina, the presence of the level of depression and the severity of depressive disorder in medical students (Fig. 1).

As a result of psychological testing, it was found that 60.0% (48 people) of the surveyed students of medical and pediatric faculties had no depressive symptoms and signs of depression. A mild level of depression (subdepressive state) was diagnosed in 22.5% (18 people) of the respondents, including 11 firstyear students and 7 fifth-year students. A mild level of depression does not require serious psychological assistance, however, informing and creating preventive measures in the early stages will help prevent the development of a depressive state and emotional disorders in students. The average level of depression (moderate depression) was found in 8.75% of medical students (7 people). It can be assumed that this result reflects the stressful state of students in the process of educational activities, the ongoing pandemic of coronavirus infection and the state of exit after self-isolation. Such a result may be a reflection of the neurotic state of the students. Severe depression (moderate) was observed in 8.75% of the respondents (7 people). This result may also be a reflection of stress and personality influences. Based on psychodiagnostic testing on a scale (test questionnaire) A.T. Beck, the students were distributed as follows:Group 1 - students who have no signs of depression in the amount of 48 people (60.0%);Group 2 - students with a mild level of depression in the amount of 18 people (22.5%); Group 3 — students with moderate and severe depression in the amount of 14 people (17.5%).

As a result of test diagnostics using the "indicator of coping strategies" technique by J. Amirkhan (adaptation by N. A. Sirota and V. M. Yaltonsky) showed (Figure 2) that for students of the first group without signs of depression, the dominant coping strategy is "problem solving "(24.8%). Medical students of the first group demonstrate a constructive approach to solving life problems and situations, which is a positive indicator of adaptation to social life (Fig. 2).

Students of the second group with a mild level of depression also prefer the productive coping strategy

of "problem solving" (23.1%), indicating that they are ready to solve emerging difficulties through dialogue with others, that is, they are focused on a productive and constructive way of overcoming difficult situations.

Among the students of the third group with moderate and severe depression, it was found out that most often they use the strategy of "avoiding problems" (23.3%). This coping strategy is the least effective attempt to get out of stressful situations. The identified coping strategy can negatively affect the psychological health of students and may be a prerequisite for the development of depressive disorder. Thus, students with moderate to severe depression use the non-constructive coping strategy of "avoidance". This coping strategy in the third group was significantly higher in comparison with the 1st and 2nd groups of respondents (p <0.05). In the questionnaire, students gave a subjective assessment of their condition, noted the absence or presence of depression. The level of depression among students was diagnosed using a scale (test questionnaire) by A.T. Beck (Beck Depression Inventory), adapted by N.V. Tarabrina. We carried out a correlation analysis of the data obtained. K. Spearman's rank correlation coefficient was used. The relationship between the subjective assessment of the state and the level of depression according to the A.T. No back was found (r=0.177; p>0.05). The obtained result of the correlation analysis indicates that students cannot adequately assess their condition and determine whether they have depression. The results obtained during the study are preliminary, and work in this area is promising and will continue.

#### CONCLUSION

Analyzing the results of the study, it should be noted that the students are familiar with the symptoms of depression and assess the impact of depression on health correctly. However, they are less informed about the treatment of depression and are biased against antidepressants. The opinion of whether depression is a disease was divided equally among the student community. It was found out that the students with moderate and severe depression most often use the non-constructive strategy of "avoiding problems" while facing stressful situations. In connection with the results obtained, it is necessary to note the importance of psychoeducation work on the treatment and prevention of depression. It is necessary to work with student youth to inform them about the risk factors, methods of preventing depression and promoting a healthy lifestyle. The results of this study will help to contribute to the differential diagnosis and prevention of reducing the negative consequences of depression.



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# AGE-RELATED MORPHOLOGICAL CHANGES IN INTIMA OF GREAT SAPHENOUS VEIN IN HEALTH AND IN VENOUS DISEASE

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ABSTRACT — AIM: To study and evaluate degree of morphological changes in intima of great saphenous vein (GSV) in patients of different age groups in health and in venous disease.

MATERIALS AND METHODS: We investigated autopsy material of GSV in 60 deceased patients and postoperative material of GSV in 80 patients with venous disease. Four age groups were formed: young age, middle age, older and elderly age. Totally there were 280 GSV fragments. Histological, morphometric, electron microscopic and biochemical studies were performed.

RESULTS: Our results are mainly consistent with the reference data. However, we carried out a comparative study of intima structure in norm and with venous disease in patients of different age. At the same time due to agerelated decline in varicose veins morphological changes are progressing. There are areas of endothelium atrophy and signs of fibrotic replacement in young and middle age patients with venous disease, whereas in older and elderly patients desquamation of endothelium and sclerosis were observed.

CONCLUSION: Progressive degeneration in older and elderly patients with varicose veins promotes morphological changes of GSV and progression of the disease on histological and ultrastructural level that worsens endothelial dysfunction.

**KEYWORDS** — great saphenous vein (GSV), intima, varicose veins, chronic venous insufficiency, degenerative changes.

### INTRODUCTION

Despite advances in medicine and science, some issues on development and pathogenesis of varicose disease (VD) still remain disputable. All over the world the prevalence of varicose veins in elderly people over 60 years of age has been increasing. The reference literature contains extensive data on morphology of venous walls; however we could hardly come across studies dealing withage-related morphological changes in intima of great saphenous vein in patients with VD.

#### Aim:

To study and evaluate degree of age-related morphological changes of GSV intima in norm and with venous disease.

#### MATERIALS AND METHODS

The autopsy material of the veins in 60 deceased patients without pathological changes in the wall of the investigated veins of the lower extremities was studied. Age groups of deceased and patients were assigned in accordance with the WHO classification: group 1 (young people); Group 2 (middle-aged people); Group 3 (older people), Group 4 (elderly people). Each group included 15 cases. In 80 patients with 4–6 clinical stages of chronic venous insufficiency (CVI) of lower extremities (CEAP classification), we estimated postoperative material of GSV and divided the fragments between four age groups. Pieces of GSV were obtained from the area located in the upper and middle third of the thigh and the saphenofemoral junction. A total of 280 fragments of GSV were examined (Table 1).

The histochemical study was carried out on paraffin sections stained with hematoxylin and eosin and by Van Gieson. We studied volume fraction (VF) of intima using a morphometric study. In 19 patients we performed ultrastructural study of 133 GSV fragments using a JEM 100 CX electron microscope (JEOL, Japan) at an accelerating voltage of 80 kV. Statistical data processing included the calculation of the arithmetic average (M), its error (m). The significance of the differences was judged by the value of the Student t-test and was considered significant at P <0.05.

### **RESULTS AND DISCUSSION**

In young patients with varicose veins intima is thicker compared to norm. Mostendothelial cells are flattened with heavily elongated cytoplasm and thin side branches. Desquamation of GSV endotheliocytes is focal. The base of thick intima consists of mesenchymal matrix with signs of fatty infiltration; rare

Groups	Normally		Venous disease	
	Number of cases	Number of GSV fragments	Number of patients	Number of GSV fragments
1	15	30	20	40
2	15	30	20	40
3	15	30	20	40
4	15	30	20	40
Total	60	120	80	160

#### Table 1. Number of histological examinations

smooth muscle cells (SMCs) and fibroblasts. During an ultrastructural study the continuity of the endothelial lining persisted. At the same time ultrastructural changes were detected in endotheliocytes which indicates dysregulation of intracellular bioenergy and reducing the activity of metabolic processes in cells. The basal membrane was unevenly thick, in most regions rather thin and discontinuous or absent. Endotheliocytes with total destruction of all intracellular organelles and membrane structures were detected. The subendothelial layer is thin, friable and composed by the connective tissue with separate SMCs that were swollen with fragments of collagen and elastic fibers.

In healthy middle-aged and older patients we observed unevenness of GSV wall thickness. In patchy regions the intima made up of a layer of endothelial cells and a very thin subendothelial layer. In thicker parts of GSV wall subendothelial layer is well expressed with friable smooth muscle cells and elastic net with elastic membrane and longirudinal layer of media externally.

There are an increase in the length of endothelium atrophy and progressing myoelastosis in VD. In intimal stroma there are separate scattered myocytes. It is worth noting that the nature of ultrastructural changes in the wall of GSV depends on the duration of the disease so in patients of middle-aged group suffering from varicose disease for 10 years, the desquamation of the endothelium was determined on a greater length of the venous wall.

In older patients there are an increase in focuses of intimal endothelium desquamation and myelofibrosis in stroma. In stromal intima prevailed connective tissue elements, SMCs and dystrophic fibroblasts. Ultrastructural intima changes in patients of this group are more pronounced and characterized by invagination atrophy lysis of destructively modified endothelial layer.

In elderly patients the thickness of intima was more than 2 times less than that of more young patients. Intima is rarely lined with elongated and highly blended endothelial cells. The elastic net is very rare and has no distinct boundaries. There are increase of endothelial desquamation of intima and preserved endotheliocytes are swollen and damaged. Ultrastructural study confirms the progression of scleroticallydegenerative changes in the form of destruction of the endothelial layer. The basal membrane at the same time was swollen, fragmented, stratified and vacuolar. There are SMCs focal lysis and the destruction of the miofilaments especially in the nuclear zone.

Quantitative analysis of the structure of the intima of the GSV wall with the help of methods of morphometry normally and in varicose diseases in people of different age groups showed that in the normal conditions despite the degenerative changes occurring in the structure of the intima the volume fraction in all age groups has practically equal values and only in senile patients have reliably insignificant (p > 0.09) decrease. In groups of young and middleaged patients a statistically reliable increase in VF of intima was found compared with the patients of elderly and senile age groups (p < 0.001). In the group of senile patients the volume fraction of intima is reduced to a greater extent (3.2 times) compared with the patients of young and middle-aged groups. (Fig. 1).



Fig. 1. VF indexes of GSV intima in people of different age groups normally and in varicose disease  $(M \pm m)$  (VFin %%).

#### DISCUSSION

According to some authors the primary cause of the venous disease of the lower limbs are morphological changes in the venous wall primarily in the intima thickness due to deregulation of the components of the connective tissue as well as changes in the structure of the media resulting in transformation of smooth muscle cells. [1, 2, 3] Other authors conclude that endothelial dysfunction plays an important role in chronic venous insufficiency (CVI). This fact is explained by disruption of the morpho-functional state of endothelial cells, which leads to an inflammatory cascade with subsequent pathological changes in the walls of the veins.[4, 5, 6, 7]

#### CONCLUSION

The results of our study are mainly consistent with the reference literature.However we focused on comparative estimate of intima structure in norm and in venous disease in patients of different age. At the same timedegenerative changes in varicose veins contribute to morphological changes that are progressingwith age. There are regions of endothelium atrophy and microelastofibrosis in young age and middle age patients with varicose veins while in older and elderly patients desquamation of endothelium and sclerosis were noted.

Thus,degenerative processes in older and elderlypatients with varicose veins affect the morphology of GSV and the disease progression on histological and ultrastructural levels, which in its turnaggravates endothelial dysfunction.

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# THE ROLE OF FINE PARTICLES THAT POLLUTE AMBIENT AIR IN ATHEROSCLEROSIS PATHOGENESIS: A LITERATURE REVIEW

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#### INTRODUCTION

The most recent report by the Global Burden of Disease (GBD), which analyzed mortality due to all causes and particular causes, stated that PM 2.5 was the fifth most important global risk factor that had caused 4.2 million deaths per year, and most of these deaths were accounted for by cardiovascular diseases (CVD) [15]. According to a statement by World Health Organization (WHO) only 18% of population are subjected to the impact of such annual average levels of fine particles (levels of fine particles (PM 2.5) that conform to the Air Quality Guidelines (AQG) (WHO, 2018) [38]. Epidemiological and toxicological studies have unambiguously demonstrated that the impact of PM 2.5 threatens human health and causes increased rate of cardiovascular diseases and mortality of human population [3, 7, 34, 39]. Atherosclerosis was a pathological foundation for practically all known CVD [2, 5, 6, 8–14]. Globally atherosclerotic CVD have long been considered the leading cause of death [2, 4, 30]. A number of published studies are indicative of the fact that PM 2.5 can promote the occurrence and progression of atherosclerosis in human organism [6, 13, 17]. The optimal content of PM2.5 in the air is  $\leq 10 \text{ mcg/m}^3$  (according to WHO), however, several studies have reported that even lower levels of PM 2.5 can be associated with atherosclerosis and CVD [5, 8, 9].

#### *The objective*

of this article is to study the impact of fine particles polluting ambient air on the development and progression of atherosclerosis and CVD, as well as identify the main pathophysiological mechanisms underpinning PM 2.5-induced atherogenesis. ABSTRACT — BACKGROUND. Atmospheric air pollution with fine particles (PM 2.5) is one of the global challenges having an impact on the disease rate, mortality, and disability of the earth's population. The optimal content of PM2.5 in the air is < 10 micrograms/m3 (according to WHO), however, this concentration is not completely safe. Currently the impact of fine particles that pollute ambient air on the progression of atherosclerosis and cardiovascular diseases (CVD) is being actively studied. Pathogenetic mechanisms enabling fine particles to partake in atherogenesis are being studied. Understanding specific fundamental mechanisms which underpin PM 2.5-induced atherogenesis allows to improve prevention-care intervention aimed to mitigate the negative impact of PM 2.5 on pathogeny of atherosclerosis and CVD. OBJECTIVE. Study of the impact of fine particles polluting ambient air on the development and progression of atherosclerosis and CVD, as well as discussion of the main pathogenetic mechanisms that underpin this phenomenon. METHODS. PubMed/Medline and Embase databases have been used for searching and analyzing modernday literature. We looked at the terms «fine particles», and «PM 2.5» in combination «atmospheric air» with «atherosclerosis». The search for literary sources has been carried out over the past 15 years. RESULTS. Many experimental and clinical studies prove the connection between fine particles (PM 2.5) polluting ambient air and the risk of atherosclerosis progression and further CVD progression, all the way through to the increased risk of unfavorable cardiovascular events (acute myocardial infarction, cerebral vascular accidents) in persons inhabiting polluted areas. PM 2.5-induced atherosclerosis is underpinned by the following pathogenetic mechanisms: Induction of oxidative stress and enhancement of inflammatory reactions, progression of endothelial dysfunction, as well as dysfunction of vegetative nervous system, and imbalance of coagulative blood system. CONCLUSION. According to the review conducted fine particles PM 2.5 play an important role in the pathogeny of atherosclerosis and CVD, and they partake in practically all the key pathophysiological mechanisms. PM 2.5-induced atherogenesis is underpinned by multiple mechanisms, the most important ones being oxidative stress and inflammation, endothelial dysfunction, dyslipidemia, dysfunction of vegetative nervous system and hemostatic system. Contribution of fine particles polluting ambient air in the progression of atherosclerosis is so much so great that it must be taken into account, and prevention-care intervention must be administered to persons residing in the most unfavorable areas.

**KEYWORDS** — Ambient air pollution, atherosclerosis, cardiovascular diseases (CVD), fine particles, PM 2.5, pathogenesis.

#### **METHODS**

PubMed/Medline and Embase databases have been used for searching and analyzing modern-day literature. We looked at the terms «fine particles», and «PM 2.5» in combination «atmospheric air» with «atherosclerosis». The search for literary sources has been carried out over the past 15 years.

#### PM 2.5 AND ATHEROSCLEROSIS

Fine particulate matters (PM 2.5) are a complex mix of microscopic particular matters in the atmosphere that vary in terms of both size and composition. Volcano eruptions, water drops, sand storms, and plant sources are the relatively few main natural sources of PM formation. At the same time, human sources are more complex and diverse. They include fossil fuel incineration, emissions by industrial facilities, smoke, road dust, and vehicle exhaust fumes. The composition of atmospheric particulates is complex and it depends on various factors, such as emission sources and weather conditions [37]. Physical and chemical properties of PM and their effect on human body are determined by various factors relating with PM, primarily the size of particles, their concentration and composition. PM can be divided into four categories in accordance with their aerodynamic diameters (AD): large particles (AD  $\leq$  10  $\mu$ , PM 10), small particles  $(AD \le 2.5 \mu, PM 2.5), PM 1 (AD \le 1 \mu), and ultrafine$ particles (AD  $\leq 0.1 \mu$ , PM 0.1). Different-size particles can have varying impact on human health. For instance, PM 10–2.5 can be deposited in respiratory system causing diseases including cough, hyper sensitivity, and bronchitis [28]. PM 2.5 can move up and down respiratory passages and enter alveoli and stay deposited in alveoli thereby causing inflammation in the lungs. The finest components, especially ultrafine particulates, are able to enter blood circulation and blood cells via the vascular barrier, which testifies to the substantially increased number of studies that evaluate the impact of high and low concentrations of PM 2.5 on human health, which demonstrate negative effect on human health due to the impact of PM 2.5, even at the levels below those recommended by WHO standards [36]. There is a 'dose-effect' relationship between PM 2.5 concentration and the ability to cause inflammatory responses and oxidative stress, which positively correlates with disease rate and mortality rate due to CVD [6, 41]. In vitro и in vivo trials also provided evidence in support of this viewpoint [3, 15]. Release of inflammatory factors during atherosclerosis progression depends on PM concentration [41, 43]. Toxicity of a certain dose of PM 2.5 and its main components is based on the concentration of pollutants in the actual milieus, as well as frequency and duration of

exposure of pollutants on human body [42, 43]. PM 2.5 composition substantially varies depending on particular areas and weather conditions which may have varying toxicological effect. At the same time, organic components of PM 2.5 can be more closely connected to CVD, while inorganic components can be more closely related to pneumopathy [25].

Although various components of PM 2.5 have varying degree of impact of human health, they nonetheless promote the occurrence and progression of atherosclerosis. A major study stated that longterm exposure to organic carbon which is part of PM 2.5 can cause the increase of Intima-media thickness (IMT) of carotid artery [20]. Another study also indicated that all the reviewed components of PM 2.5 were related with increased IMT of carotid arteries, and that organic carbon had the highest pathogenetic effect [32]. A recent study conducted in China stated that each increase of iron concentration by 0.51  $\mu/m3$ and nickel-iron by 2.5  $ng/m^3$  in PM 2.5 resulted in the increased of oxidized LDL (ox-LDL) in blood by 1.9% (95% CI 0.2%-3.7%) and 1.8% (95% CI 0.2%-3.4%), correspondingly, which allows to suggest that the metal component of PM 2.5 can promote enhancement of oxidative stress related to PM 2.5-induced atherosclerosis [40].

#### THE KEY PATHOGENIC MECHANISMS OF PM 2.5 — INDUCED ATHEROSCLEROSIS

Oxidative stress and inflammation. PM 2.5 depositing in bronchi and alveoli after inhalation causes local and systemic inflammation, as well as oxidative stress, which as it has been widely acknowledged is one of the most important mechanisms favoring atherosclerosis [8, 10]. As PM 2.5 is a complex mix comprising a multitude of different aldehydes, polycyclic aromatic hydrocarbons (PAHs), transition metals, and other organic and inorganic components, they can enter into various chemical reactions, both between themselves and between chemical components of a human body. When these substances enter human body they are able to change initial oxidation-reduction homeostatic body state and oxidation-reduction processes by way of generating active oxygen species and nitrogen (AOS/ANS)[16, 35]. Inflammatory reaction and oxidative stress promote and enhance each other, promoting a number of reactions including generation and breakage of atherosclerotic patches. Thus PM 2.5 can indirectly promote and occurrence and progression of atherosclerosis via oxidative stress and inflammatory reaction.

*Endothelial dysfunction*. Endothelium damage is widely acknowledged as the initial event for

the progression of atherosclerosis. Endothelial cells release endothelial factors that cause constriction and relaxation of blood vessels and maintain balance between them using a complex regulatory mechanism. Reactive hyperemia, flow-mediated vasodilatation (FMD), vasoconstriction and baseline arterial diameter (BAD) are sensitive indicators of endothelium function [24]. An extensive incidence study showed that long-term exposure to PM 2.5 can substantially degrade endothelium function due to the reduction of FMD and enhancement of vasoconstriction [21]. In vivo and in vitro trials also provided the evidence of progression of PM 2.5-induced damage of endothelial cells and disruption of their normal operation. It has been demonstrated that dysfunction of endothelial cells caused by PM 2.5 was mostly implemented via indirect cytotoxic effected as caused by inflammatory cytokines and oxidative stress [29]. Thus, PM 2.5 can initiate damage and dysfunction of endothelial cells thereby causing a number of pathophysiological reactions in atherogenesis.

*Lipid storage disease*. Lipid storage disease is yet another important risk factor for atherosclerosis progression [10, 12, 17]. Randomized double blind cross-sectional study showed that the impact of PM 2.5 had resulted in significant changes in whey metabolites, including hormones, glucose, amino acids, and lipids [22]. Recent studies demonstrated that the impact of PM 2.5 can cause dyslipidemia, including the increase of concentration of total cholesterol, LDL and ox-LDL, levels of chylomicrons and triglycerides, as well as reduction of the level of antiatherogenic particles (high density lipoproteins (HDL)) [23]. These studies provided compelling evidence that PM 2.5 can accelerate congestion of lipids in plates changing the metabolism of lipids and the properties of lipoproteins, such as stimulation of acidification of LDL and their transformation into more atherogenic particles of ox-LDL.

**Disruption of vegetative nervous system**. Abnormal agitation of vegetative nervous system is one of the main mechanisms of malfunction of the cardiovascular system caused by PM 2.5. Parameters, such as heart rate, heart rate variability (HRV), and arterial tension, are governed by vegetative nervous system. HRV is a marker of vegetative nervous system of the heart [1]. Epidemiological studies showed that the impact of PM 2.5 can disrupt operation of vegetative nervous system of the heart, as well as change HRV [19]. The main mechanism lies in the fact that excessive oxidative stress and systemic inflammation aggravate the negative impact of PM 2.5 on cardiac vegetative function resulting in the reduction of HRV [27]. Besides, the impact of PM 2.5 can also cause dysfunction of sympathetic nervous system causing drastic variation of arterial tension. Elevated blood pressure can stimulate platelet-derived growth factor (PDGF) expression which, in turn, additionally induces proliferation of smooth muscle cells [27, 28]. In addition, hypertension can give rise to the formation of fibrous capsules and cause breakage of atherosclerotic patches [6, 10, 14].

Disruption of hemostatic system. Yet another possible mechanism of progression of atherosclerosis and CVD is agitation of hemostatic system caused by PM 2.5. Platelets and various coagulating albumens take part in the regulation of occurrence and development of atherogenesis and CVD by regulating inflammation, acidification, and immunoreactions. The impact of PM 2.5 is strongly correlated with changes in the concentration of blood coagulation markers, including fibrinogen, endogenic thrombin, tissue plasminogen activator (t-PA) and inhibitor of plasminogen activator-1 (PAI-1) in human blood serum, which is proves the connection between the hypercoagulation condition and the impact of PM 2.5 [18]. It has been shown that PM 2.5 inhibited t-PA and increase the release of PAI-1 from endothelial cells. Besides, PM 2.5 accelerates the formation of arterial thrombus due to enhanced agitation of platelets and aggregates of platelets-monocytes [33]. Long-term exposure of patients to high concentrations of PM 2.5 can result in the increased risk of progression of venous thrombosis and changes in the structure of atherosclerotic patches thereby increasing the probability of their breakage [26].

#### CONCLUSION

In view of the above ambient air pollution by fine particles (PM 2.5) must be considered one of the key risk factors in the development and progression of cardiovascular diseases. Of most concern is the information about excessive norms of impact of PM 2.5 on human body. PM 2.5-induced atherogenesis is underpinned by multiple mechanisms, the most important ones being oxidative stress and inflammation, endothelial dysfunction, dyslipidemia, dysfunction of vegetative nervous system and hemostatic system. Understanding specific pathophysiological mechanisms allows to recommend patients residing in the most unfavorable areas in terms of PM 2.5 pollution to allow prevention-care intervention aimed at restricting the negative impact of PM 2.5.

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# CHARACTERISTICS OF THE INTERACTION BETWEEN LOW-INTENSITY LASER RADIATION AND ERYTHROCYTES

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ABSTRACT — The results of the effect of low-intensity laser radiation on lipid peroxidation and the sorption capacity of erythrocyte membranes under stress are presented. The membrane-stabilizing effect of low-intensity laser radiation and a decrease in the rate of free radical processes are noted, which indicates the prospects of using laser radiation as an activator of the body's natural antioxidant systems. An increase in the sorption capacity of erythrocyte membranes proves the advisability of using low-intensity laser radiation to increase the adaptive capacity and to protect the body from stress.

**KEYWORDS** — low-intensity laser radiation, stress, lipid peroxidation, sorption capacity of erythrocyte membranes, adaptation.

#### INTRODUCTION

Currently, the problem of the impact of stress on the body is becoming more relevant. A promising approach to solving this issue may be the use of low-intensity laser radiation (LLLT). The available experimental studies indicate that the LLLT effect is based on a complex nonspecific effect on the body, when local changes cause a change in the functioning of bio systems due to the formation of a protectiveadaptive reaction. Despite this, the introduction of LLLT into clinical practice is mainly empirical; there is practically no experimental justification for the use of laser light [2, 3, 5].

The blood system, being one of the main homeostatic systems of the body, plays an important role in the formation of adequate compensatory-adaptive reactions under stress. As a marker of adaptive reactions, erythrocytes are used, structural rearrangements, which are an integral reflection of the reaction of cells at the level of the whole organism [1, 7].

These circumstances determined the purpose of the study - to estimate the effect of low-intensity laser radiation on the functional characteristics of erythrocyte membranes under stress.

#### MATERIAL AND METHODS

4 groups of animals were formed. Animals of the first group served as control (intact), animals of the second group exposed to LLLT, animals of the third group exposed to technological stress, and animals of the fourth group exposed to LLLT for 5 minutes in the area of the withers against the background of stress. A laser therapeutic complex - an autonomous laser shower "MarsIK" (NPO "Petrolaser", St. Petersburg) with a radiation wavelength of 890 nm was used as a radiation source.

The sorption capacity of erythrocyte membranes (RBC) evaluated by the intensity of sorption of erythrocyte membranes of methylene blue [6], the functional state of erythrocytes was judged by the accumulation of malondialdehyde (MDA), the content of which was assessed by the method of M.S. Goncharenko and A.M. Latypova [4]. Blood sampling performed 7, 14, and 30 days after the start of the experiment.

Experiments with animals wereprovided in accordance with the rules of the European Convention ET/S 129, 1986 and Directives 86/609 EEC.

Statistical processing carried out using the BIO-STAT and Microsoft Excel software package. To assess the significance of differences between the groups, Student's t-test was used (p < 0.05).

#### RESULTS

Because of the study, it found that the intensity of lipid peroxidation processes under stress undergoes significant changes over a long period. A significant increase in the concentration of MDA in erythrocytes noted by the 7th day; by the 14th day, the tendency towards an increase in the MDA level remained.

The effect of LLLT on stressed animals by day 7 determined the preservation of an increased level of MDA in erythrocytes, revealed during stress. 14 days after exposure, the level of MDA in erythrocytes was lower relative to the group of animals that underwent stress and approached the value of intact animals. 30

days after stress in all groups, the MDA content restored to the level of intact values.

The data obtained are shown in table 1.

To assess the state of erythrocyte membranes, the sorption capacity of erythrocytes studied using methylene blue. Analysis of the sorption capacity of erythrocytes provides information on changes in the barrier properties of the plasma membrane. The decrease in the sorption capacity interpreted from the standpoint of the energy deficit in erythrocytes. An increase in the sorption capacity of erythrocytes considered an indicator of membrane damage and cellular disorganization [5].

As shown by the studies presented in table 2, under stress on the 7th day, an increase in the sorption capacity of erythrocytes observed. On the 14th day after stress, the value of the sorption capacity of erythrocyte membranes decreased relative to the values of the control group.

The impact of LLLT against the background of stress promoted an increase in the sorption capacity of erythrocytes by the seventh day. By the 14th day, the sorption capacity of erythrocyte membranes approached that of the control group.

Thus, the effect of stress is manifested by an increase in the processes of free radical oxidation and, accordingly, changes in the lipid spectrum of the erythrocyte membrane. This leads to some loss of peripheral low molecular weight membrane proteins and a relative increase for glycoproteins, which, apparently, increases the sorption capacity of erythrocytes. When a certain critical level of the protein-lipid ratio in the erythrocyte membrane reached, there is already a loss of integral proteins, which leads to a decrease in the sorption capacity of erythrocytes [8].

#### CONCLUSION

The use of LLLT reduces the content of LPO products, normalizes the sorption capacity of erythrocyte membranes. The LLLT effect is based on its ability to stimulate various processes of protection, adaptation, and compensation. The electromagnetic nature of LLLT suggests the possibility of its interaction with a variety of regulatory mechanisms in living systems. The most important regulatory system of the body is the system of free radical processes, which is associated with many biological phenomena, including the mechanisms of regulation of membrane permeability [9].

Thus, the experimental data give reason to believe that the use of LLLT under stress is justified. 
 Table 1. Malonicdialdehyde content (nmol / ml) in erythrocytesunder stress and exposure to low-intensity laser radiation

Group of animals	7 day	14 day	30 day
Control (intact animals)	1.72±0.23	1.91±0.31	1.85±0.26
Technological stress	2.74±0.29*	2.29±0.25*	1.92±0.31
Control + LLLT	1.78±0.26	1.85±0.42	1.91±0.23
Stress + LLLT	2.61±0.35*	2.12±0.37	1.87±0.29

*Note:* \* — *p* < 0.05 relative to the control group

**Table 2.** Sorption capacity of blood erythrocytes under stress and exposure to low-intensity laser radiation, %

Group of animals	7 days	14 days	30 days
Control (intact animals)	36.2±1.8	39.7±1.5	38.5±1.4
Technological stress	62.4±1.5*	54.4±1.5*	40.3±1.2
Control + LLLT	32.7±1.4	36.1±1.8	36.2±1.3
Stress + LLLT	52.6±1.9*	42.3±1.4*	34.7±1.9

*Note:* \* — *p* < 0.05 relative to the control group

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# **EFFECT OF SOME BIOCHEMICAL FACTORS ON FEMALE REPRODUCTIVE FUNCTION**

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ABSTRACT — The study aimed to determine the effect of certain biochemical factors on the female reproductive function. Analysis showed that two biochemical factors classified as inflammatory markers have a significant difference in their concentrations in women with impaired fertility and those without fertility disorders. These are C-reactive protein (CRP) and lactoferrin (LF). It is reasonable to assume that these inflammatory markers are associated to some extent with the development of reproductive disorders due to possible formation of inhibin A/LF complexes, which may reduce the biological activity of inhibin A, in patients' serum. The effect of CRP on inhibin A activity is most likely to be systemic and associated with the effect of CRP high concentrations on inhibin A function on the body level. In particular, it is possible that CRP has an effect on IL-1ßsynthesis in ovarian follicles.

**KEYWORDS** — C-reactive protein, lactoferrin, interleukins, fibrinogen,  $\alpha 2$  macroglobulin reproduction.

#### INTRODUCTION

According to a number of researchers, an inflammatory process in patients, accompanied by a number of biochemical factors in the serum, may reduce the relevance of sufficient levels of inhibin A, belonging to the group of Smads [2]. Inhibin A is a protein secreted by ovarian granular cells, among others, and it has an effect on the secretion of follicle-stimulating hormone (FSH). Inhibin A is present in blood plasma and in follicular fluid in large quantities. Its main function is to inhibit follitropinsecretion by the pituitary gland by affecting the hypothalamus [7, 11].

Factors that cause a decrease in the biological activity of inhibin A primarily include cytokines and acute-phase proteins. Finding out the causes of a decrease in the level and activity of inhibin A in women with inflammatory diseases of the reproductive system, including infectious diseases, will both improve the quality of diagnosis and provide new opportunities for the development of fertility treatment methods [1, 3, 8, 10].

The study aimed to determine the effect of certain biochemical factors on the female reproductive function. **MATERIALS AND METHODS** 

The study is focused on the blood serum of women with chronic inflammatory processes, in particular chronic trichomoniasis. Blood serum of almost healthy fertile women having no trichomoniasis, including in past medical history, was used as a control.

160 women with chronic trichomoniasis were examined. They were from 22 to 29 years old, with the average age of 24.1+3.45.

The level of inhibin A in the blood serum of all the examined women was determined with the method of enzyme immunoassay on days 3–5 of the menstrual cycle, as recommended by WHO. 35 healthy fertile donor women without trichomoniasis and with no history of STDs (the average age of 24.3+1.41), were examined as a control.

The phenotype of lymphocytes was determined using a fluorescent microscope with monoclonal antibody straining (produced by State Research Center Institute of Immunology, Moscow, Russia).

Immunoglobulin levels in the serum were measured with the standard method of radial immunodiffusion (Mancini method), for which monospecific antisera were used (National Research Center for Epidemiology and Microbiology named after Academician N.F. Gamaleya, Moscow, Russia). The content of immunoglobulins was expressed in mg/%.

Cytokines were produced through spontaneous synthesis of IL-1 $\beta$ , IL-8, IL-10 and IFN $\gamma$ . Lymphoid cells were preincubated for 24 hours. The concentration of the above cytokines was determined with immunoenzyme method. Concentrations of IL-1 $\beta$ , IL-8 were determined using test kits produced by Protein Contour (St. Petersburg), the ones of IL10 and IFN $\gamma$  – with Immunotech test kits (France).

Lactoferrin was determined with immunoenzyme method using test kits produced by Cytokine company (St. Petersburg).

Concentrations of a2-macroglobulin and Creactive protein were determined with nephelometric method. Fibrinogen concentration was determined with coagulological method.

#### RESULTS

Levels of IFN $\gamma$ , IL-8 and IL-1 $\beta$  were determined to describe the functional state of Th1 and Th2 in the patients. These cytokines are universal regulators of both inflammatory responses and the whole immune system, with a wide range of biological effects including antibody formation, proliferation of T and B lymphocytes and induction of synthesis of other cytokines [4, 5, 9].

In vitro production of IL-1 $\beta$  by mononuclear cells in the healthy women averaged 102.6±8.4 pg/ml and ranged from 96.1 to 111.0 pg/ml. The rate of spontaneous IL-1 $\beta$  production in the patients with chronic trichomoniasis was significantly higher and equaled to 292.7±12.1 pg/ml (p<0.001). Therefore, it becomes evident that there is definite correlation between the degree of IL-1 $\beta$  production and trichomoniasis infection.

IL-8 is known as a classic pro-inflammatory cytokine that induces an acute inflammatory response, increasing the adhesive capacity of neutrophils. While its concentration in the healthy women was  $19.7\pm2.3$  pg/ml, the rate of spontaneous production of IL-8 in the patient group was lower (p<0.001)than the reference values, being only  $10.9\pm2.1$  pg/ml, which suggests a decrease in the phagocytic activity of neutrophils, associated with it.

The level of IFN $\gamma$  is equally important for normal functioning of the immune system; as known, it regulates immune response intensity and intensifies the expression of antigens belonging to classes I and II of the major histocompatibility complex. IFN $\gamma$  stimulates bactericidal activity of macrophages and neutrophils by activating oxygen-dependent and oxygen-independent mechanisms. Besides, this mediator also influences the functional state of NK cells, the first line of protection against viral and bacterial infections. It was determined that the level of IFN $\gamma$  in the healthy women was 25.10±1.60 pg/ml, while spontaneous production of IFN $\gamma$  in the patients with trichomoniasis was almost three times lower and equaled to 8.3±1.1 pg/ml (p<0.01).

Another important immunoregulatory cytokine is IL-10, an anti-inflammatory agent produced by Th2. It is considered that its biological function is mainly to inhibit IFN $\gamma$  production. Spontaneous production of IL-10 in the group of healthy women was from 114.0 to 250.0 pg/ml (178.3±41.5 pg/ml on average). Its concentration in the patients was evaluated — up to 1564.0±123.0 pg/ml, i.e. more than 8-fold (p < 0.001). It is obvious that the lack of IFN $\gamma$  production, on the one hand, reflects the impaired functional activity of CD4+ cells in the patients, and, on the other hand, may lead to infection tolerance.

The content of immunoglobulins IgA and IgM in the patients was within normal limits, though there were changes in their ratio (as a rule, an increase in the level of IgA and/or decrease in the level of IgM), which was identified in two thirds of the patients.

Therefore, examination of the cytokine profile in the women with chronic trichomoniasis clearly demonstrates prevailing development of the Th-2 type immune response.

It is known that cytokines initiate synthesis of secondary mediators of inflammation — acute-phase proteins — by hepatocytes. These include, among others, plasma glycoproteins produced by the liver in response to tissue damage. Plasma proteins are divided into several classes depending on the increase in their concentrations in case of acute-phase states. A decision was made to examine concentrations of lactoferrin (LF), C-reactive protein (CRP),  $\alpha$ -2-macroglobulin and fibrinogen. According to the authors' data, the level of CRP in the group of healthy women was  $0.3\pm0.01\mu$ g/ml. Its concentration in the patients showed more than 65-fold increase — up to  $19.5\pm0.8 \,\mu\text{g/ml} (p < 0.001)$ , but this is much lower than in most bacterial infections and more typical of chronic viral diseases.

The level of lactoferrin in the group of healthy women under examination was  $445.0\pm104.0$  ng/ml. Its concentration in the patient group was elevated almost 8-fold – up to  $3,475.0\pm178.0$  ng/ml (p < 0.001). Such values are more often seen in acute infections (including sexually transmitted diseases) and are not typical of most chronic bacterial infections at all, although bacteria are capable of synthesizing protein that is immunochemically similar to human lactoferrin [6, 10].

Then the authors performed a retrospective analysis of variation series, with the results obtained being grouped regardless of the concentration of inhibin A and the inflammatory factors under study, in their relationship with the state of the reproductive function of the women having trichomoniasis. As mentioned above, infertility was observed in 30 women in the patientgroup with normal levels of inhibin A, while the rest kept fertile or had no reliable data on fertility disorders. The conducted analysis showed that only two inflammatory markers in the blood of the women having a chronic inflammatory process have a significant difference in their concentrations in the women with impaired fertility and those without fertility disorders. These are C-reactive protein and lactoferrin.

It is natural to assume that these inflammatory markers are associated to some extent with the development of reproductive impairment in the women suffering from trichomoniasis. The authors admit possible formation of inhibin A/LF complexes in the patients' serum which may reduce the biological activity of inhibin A. This is quite possible in at least two ways. Firstly, it is electrostatic interaction of several molecules of inhibin A, having a sufficiently high negative charge, with LF polycation. Secondly, there may be a mechanism of mostly hydrophobic interaction between LF molecules and inhibin A.

The CRP effect on inhibin A activity can hardly be explained with formation of complexes. They are most likely to be systemic and associated with the effect of CRP high concentrations on inhibin A function on the body level. In particular, it is possible that CRP has an effect on IL-1β synthesis in ovarian follicles. It is known from literature that this cytokine is synthesized by the granulosa cells and that high concentrations of IL-1 $\beta$  in the follicular fluid correlate with the degree of maturity and with the frequency of oocyte fertilization [1, 3, 11]. On the other hand, IL-1 $\beta$  is a pro-inflammatory cytokine that plays an essential role in inflammatory response unfolding. It can induce most of its local and general manifestations. It is also known that high concentrations of CRP (10  $\mu$ g/mL and more) can inhibit IL 1 $\beta$  synthesis along with prostaglandin E2 and glucocorticoids.

#### CONCLUSION

Therefore, the study results can be summed up as follows. The group of patients with a chronic inflammatory process demonstrate significant variability in the concentration of inhibin A (4.3 - 29.4 ng/l). Secondary infertility was diagnosed in 63.2% of cases in the group of patients with reduced levels of inhibin A, while the group of patients with normal levels of inhibin A demonstrated only 12.9% of cases of secondary infertility development. It was found out that C-reactive protein and lactoferrin were the only acute-phase proteins that had a prognostic value and differed significantly in the groups of women with subsequent infertility and those with a chronic inflammatory process that was not complicated with infertility.

The authors declare no conflict of interest in the manuscript being submitted.

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## CORRECTION OF IMMOBILIZATION STRESS BY MELATONIN IN RATS UNDER CONDITIONS OF DARK DEPRIVATION Received 6 October 2021; Received in revised form 27 October 2021; Accepted 29 October 2021;

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**ABSTRACT** — In this study, we have evaluated the effectiveness of melatonin in correcting stress in rats that occurred after dark deprivation and mobility restriction. The main group of animals (n=6) received melatonin in order to overcome stress. The obtained indicators for assessing the stress level (vertical activity, freezing, the number of boluses) in the main group of rats were compared with the indicators of the control (animals received placebo, n=6) and intact (n=6) groups. As a result, when the daylight lengthens, rats develop stress, which is manifested by a change in the activity of the animal. We have proved that the use of melatonin reduces hyperactivity in rats by 1.5 times (p<0.01) and the duration of freezing is reduced by  $\frac{1}{3}$  (p<0.05).

 $\overline{C}$ ONCLUSION: In animals treated with melatonin, physical and vegetative activity was significantly higher compared to the placebo group. Therefore, melatonin helps to overcome immobilization stress in rats under conditions of dark deprivation.

**KEYWORDS** — Stress, melatonin, dark deprivation, activity disorder, circadian rhythm, desynchronosys.

## INTRODUCTION

Nowadays, a person's professional activity is often associated with the need to change time zones. Certain jobs require constant presence in conditions of roundthe-clock illumination, especially for night shift workers [1]. Circadian disorders are also inherent in people living in the polar day zone, as well as those who make frequent business trips with a change of time zones. In such cases, desynchronization of the daily biorhythms of physiological functions develops in the human circadian system [2].

Desynchronosis is one of the most powerful stress factors for all body systems. With frequent occurrence and prolonged course, it can weaken the circadian organization of a person and lead to the development of pathological processes in a particular physiological system [3]. The development of desynchronosis is accompanied by a decrease in the night peak of melatonin levels, which occurs due to a violation of circadian rhythms. Therefore, the presence of the correct rhythm of melatonin production is a necessary condition for a healthy body. It is known that people experience stress when moving to work from the middle latitudes to the Far North due to a violation of the seasonal rhythm of melatonin secretion. This is confirmed by the facts of an increase in depressive states and alcoholism in this contingent.

Aim:

to evaluate the effectiveness of melatonin in the correction of immobilization stress in rats in a situation of dark deprivation.

# **METHODS**

The object of the study were 18 sexually mature white rats weighing from 200 to 400 g, contained in standard vivarium conditions. All procedures with animals were performed according to the Guidelines for the Care and Use of Laboratory Animals. We divided all the animals into 3 equal groups: 1 group (main, n=6), 2 group (control, n=6), 1 group (intact, n=6).

The animals of groups 1 and 2 were in dark deprivation conditions for 10 days. Then we placed all the rats of these groups in immobilization chambers for one hour to create a model of a stress reaction. The animals of groups 1 and 2 were in stress mode for 10 days. At the same time, we administered melatonin at a dose of 1 mg/kg intramuscularly to all rats of group 1 during this period, and we gave an intramuscular injection of 0.9% NaCl solution as a placebo to animals of group 2.

The rats of the third group formed an intact group, we did not perform any manipulations with them and were kept on a rational diet throughout the entire time of the experiment.20 days after the end of the experiment, we evaluated the following indicators in all animals of the three groups: vertical activity (the rat taking a vertical position), freezing (freezing in place), the number of bolluses (the act of defecation). We took the sign of "high motor activity" in rats as normal and interpreted it as the absence of anxiety and stress in a particular animal. Statistical processing was carried out with the calculation of arithmetic mean values (M) and their errors (m). The reliability of differences in the groups was calculated using the Mann-Whitney test. The differences were considered significant if p<0.05.

# RESULTS

The indicators of motor activity, namely vertical activity, in the rats of the first group were 1.5 times lower compared to the intact group (p < 0.01) (Fig. 1). This fact indicates that dark deprivation and impaired mobility leads to stress. However, we found that the vertical activity in the group of rats treated with melatonin was significantly higher by 22% compared to the intact second group (p < 0.05) (Fig. 1). This means that anxiety melatonin helps to reduce stress levels and helps to neutralize anxiety disorders in the animal.



Fig. 1. Vertical activity in rats of the studied groups

Another indicator of stress is freezing (freezing of the rat without movement). We noticed that the duration of freezing during one hour of observation in the first and second groups of animals was more than ten times higher than in the intact animals (Fig. 2) (p<0.001). However, against the background of the use of melatonin, the activity of rats improves, and the duration of freezing decreases by 33% (p<0.05).



Fig. 2. Indicators of freezing in rats under immobilization stress after dark deprivation

The number of boluses reflects the state of the autonomic nervous system. Therefore, the more boluses there are, the more pronounced the stress reaction in the animal. The highest number of boluses per day was in the experimental group of rats receiving placebo — 24.1+1.7. This indicator had significant differences with the group of animals receiving melatonin — 16.5+2.3 (p<0.05). In the animals of the intact group, the number of boluses per day was minimal (7.8+1.1), but there were no significant differences with the first group(p>0.05).

## DISCUSSION

Circadian rhythms are important for the normal development and functioning of the nervous system. Violations of circadian rhythms suppress the function of the nervous system and lead to the formation of stress, which has been proven in animal models [4]. The systemic response to stress, aimed at eliminating or reducing stress, is accompanied by changes in behavioral, vegetative, motor, sensory, cognitive and other functions of the body [5].

It is known that melatonin contributes to the modulation of circadian rhythmicity. Melatonin is also involved in the regulation of blood pressure and the autonomic cardiovascular system, the regulation of the immune system, as well as in various physiological functions [6]. Melatonin synthesis and secretion are enhanced in the dark and suppressed by light. Low concentrations of melatonin are usually found in patients with neurological diseases and mental disorders [7]. Positive results of the use of melatonin were obtained in the correction of mental and behavioral disorders, such as attention deficit hyperactivity disorder. Melatonin has an effect on the organs of the gastrointestinal tract. Arendt J. et al. It was noted that melatonin protects the gastrointestinal tract from ulcers by reducing the secretion of hydrochloric acid and increasing the secretion of bicarbonate of the duodenal mucosa [1]. Melatonin also promotes the regeneration of the epithelium of the intestinal mucosa. These factors indicate the properties of melatonin to correct the state of the autonomic nervous system.

Our study demonstrates that animals that are in conditions of round-the-clock illumination and impaired mobility experience stress. This stress reaction can be successfully compensated by administering melatonin to animals. We noted that the vertical activity in the main group of rats improved by 1.3 times, the number of freezing decreased by 1.5 times, and violations of vegetative reactions were practically leveled.

## CONCLUSION

Light desynchronosis leads to the development of stress and anxiety-depressive disorders. The restora-

tion of circadian rhythms helps to reduce stress, helps to maintain the balance of higher nervous activity, which contributes to the correction of behavior, mood and intellectual functions of an individual. We proved that animals treated with melatonin had significantly higher physical activity, and anxiety indicators were lower compared to the placebo group. This means that melatonin contributes to the successful overcoming of the consequences of induced stress, burdened with desynchronosis in rats.

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P H A R M A C O L O G I C A L S T U D I E S

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# INFLUENCE OF HESPERIDIN ON SOME BIOCHEMICAL INDICATORS IN MICE IN SETTING OF EXTRACTING PHYSICAL LOADING

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ABSTRACT — A study was carried out on the effect of hesperidin, administered at a dosage of 100 mg / kg, on biochemical parameters in mice in the setting of exhausting physical exertion. The change in biochemical parameters was assessed after the «Treadmill» test, which was carried out during 5 days. After that, blood was tested for further assessment of the following markers of fatigue: lactic (LA), pyruvic acid (PA), and myoglobin. These compounds were determined using a standard set of reagents. It was found that against the background of intragastric administration of the studied flavonoid hesperidin in relation to the negative control group, there was a significant decrease in lactate level by 2.3 times (p < 0.05), an increase in pyruvate by 8.8 times, a decrease in the lactate / pyruvate 21.2 times (p < 0.05). It should also be noted that the myoglobin level was lower at 2.0 (p < 0.05) in comparison with the negative control group. There were no significant differences between the group that received hesperidin during the experiment and the group of mice that received Mexidol in the above parameters (LA, PA, LA / PA, myoglobin). The results of the study obtained allow us to recommend this compound as a corrector of biochemical shifts that can occur during exhausting loads.

**KEYWORDS** — exercise, acidosis, pyruvate, myoglobin, mice, hesperidin.

# INTRODUCTION

Today, the modern level of development of sports makes special requirements for the level of fitness of athletes [1]. It is known that extreme physical activity leads to a breakdown of adaptive capabilities, which is reflected in the manifestation of glycolysis activation and the possible accumulation of underoxidized products in the body, to the development of endothelial dysfunction, as well as changes in the cardiovascular system [2]. All these factors can both directly and indirectly affect the energy potential of the body. In this regard, search for compounds capable of correcting the functional state of the body under conditions of exhausting physical exertion becomes urgent.

#### **Objective:**

to study the effect of hesperidin on some biochemical parameters in mice during exhausting physical exertion.

## MATERIALS AND METHODS

The experiment was carried out in accordance with the "Guidelines for the conduct of preclinical studies of drugs, ed. A.N. Mironov (2012 ed.) [3]. The animals were kept in the vivarium of the PMFIbranch of the Volgograd State Medical University (Volgograd, Russia). The study was carried out on 40 outbred white mice (m = 20-24 g). The animals were divided into 4 groups (n = 10). All experimental animals, in the course of the experiment, were kept in standard vivarium conditions (natural mode of light change, temperature, relative humidity, standard diet of laboratory animals, weekly change of bedding and cages, fixed feeding and drinking times) in compliance with the International Recommendations of the European Convention on protection of vertebrates used in experimental research. Previously, the animals were randomized by weight and running speed in the «Treadmill running» test. After that we formed 4 equal experimental groups. The group of positive control (PC) mice underwent physical exertion with days of rest, the second group - negative control (NC) received 0.9% sodium chloride solution throughout the entire length. The third group of animals received hesperidin at a dosage of 100 mg / kg. The fourth group received the reference drug Mexidol in a dosage [4]. All test compounds were administered intragastrically 30 minutes before testing before animals started running.

Physical activity was assessed in the treadmill test for 5 days. After that, blood was taken from the animals for further assessment of the following markers of fatigue: lactic (LA), pyruvic acid (PA), and myoglobin. These compounds were determined using a standard set of reagents manufactured by Arbis + by the enzymatic colorimetric method. Myoglobin was determined on a Tecan Infinite F50 microplate reader (Austria) by a highly sensitive enzyme-linked immunosorbent assay. The results were processed using the STATISTICA 6.0 software.

## RESULTS

In the setting of physically exhausting loads to which the animals were subjected, by the end of the fifth day of the experiment «Running on the treadmill» in the group of negative control mice, an increase in the level of lactate, a decrease in pyruvate and an increase in the lactate / pyruvate ratio, respectively, by 4.6 times (p < 0, 05) (Fig. 1), 5.7 (p < 0.05), 26.4 times (p < 0.05), in comparison with the group of positive control animals. It should also be noted that this group also had a significantly higher myoglobin level by 1.9 times.

Probably, the data obtained may indicate the development of acidosis in the body of mice, which is also accompanied by damage to the tissues of working muscles, which is confirmed in studies conducted earlier [5].

Against the background of intragastric use of the plant object hesperidin, a positive effect on some markers of fatigue is observed, which is reflected in a decrease in lactate levels by 2.3 times (p < 0.05) (Fig. 1), an increase in pyruvate by 8.8 times, a decrease in the lactate / pyruvate ratio by 21.2 times (p < 0.05), as well as a decrease in the level of myoglobin by 2.0 (p < 0.05) (Fig. 3) in relation to the negative control group.

Elimination of the acidosis process and a decrease in the marker of muscle destruction was observed in the group of animals treated with Mexidol, in comparison with the group of negative control animals. This was reflected in a decrease in LA (3.1 times (p < 0.05), an increase in PA (5.2 times (p < 0.05)), a decrease in LA / PA ((16.3 times (p < 0.05)) and a decrease in myoglobin (2.0 times (p < 0.05)).

It is also worth noting that acute anaerobic stress in the group of animals treated with the reference drug Mexidol did not lead to statistically significant changes in lactate, pyruvate, and myoglobin as compared with the group receiving the studied flavonoid hesperidin.

# CONCLUSION

Intragastric administration of the studied flavonoid hesperidin led to a significant decrease in lactate levels by 2.3 times (p < 0.05), an increase in pyruvate by 8.8 times, and a decrease in the lactate / pyruvate ratio by 21.2 times (p < 0.05), and to a decrease in the level of myoglobin in 2.0 (p < 0.05) in comparison with the negative control group. There were no significant differences between the group that received hesperidin during the experiment and the group of mice that



*Fig. 1.* Changes in the level of lactic acid in the blood serum of mice after physical exertion.

**Note:**  $\mu$  — reliably relative to the PC group (Student's t-test, p < 0,05); \* — reliably relative to the NC group (Student's t-test, p < 0,05)



# *Fig. 2.* Changes in the level of pyruvic acid in the blood serum of mice after physical exertion

**Note:**  $\mu$  — reliably relative to the PC group (Student's t-test, p < 0,05); \* — reliably relative to the NC group (Student's t-test, p < 0,05)



**Fig. 3.** Changes in the level of myoglobin in mice after physical exertion. **Note:**  $\mu$  — reliably relative to the PC group (Student's t-test, p < 0,05); \* — reliably relative to the NC group (Student's t-test, p < 0,05);

received Mexidol in the above parameters (LA, PA, LA / PA, myoglobin).

The results of the study obtained allow us to recommend this compound as a corrector of biochemical shifts that can occur during exhausting loads.

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# EVALUATION OF THE EFFECT OF THE PIR-12 COMPOUND ON ANXIETY AND LOCOMOTOR ACTIVITY IN EXPERIMENTAL GLOBAL CEREBRAL ISCHEMIA IN RATS

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ABSTRACT — A study was carried out to assess the effect of a new pyrimidine derivative (PIR-12 50 mg/kg) on anxiety and locomotor activity under conditions of global cerebral ischemia in rats. It was confirmed that the investigated compound PIR-12 helps to reduce the degree of anxiety, which is manifested in an increase in the indicators "Time at the central site" and "Time in the open arms of the maze" in 3,2 (p<0,05) and 3,1% (p<0,05). "Time in the closed arms of the labyrinth" and "the number of grooming acts" decreased against the background of the introduction of PIR-12 by 28,9% (p<0,05) and 4,1 times (p<0,05) compared with the control group animals, which surpasses the effect of the reference drug Cavinton. In addition, PIR-12 promotes an increase in the motor activity of ischemic rats: the number of hanging and standing rats was 7,4 times higher (p<0,05), 4,2 times (p<0,05), relative to the NC rat group, and the effect is comparable to the group of animals that received Cavinton.

**KEYWORDS** — cerebral ischemia, level of anxiety, locomotor activity, pyrimidine derivatives.

# INTRODUCTION

Vascular diseases of the brain continue to be one of the most important problems in modern health care, since the incidence, disability and mortality from this nosology remain at a high level throughout the world [1]. Ischemia-induced disorders of cerebral circulation, pathophysiological and pathobiochemical processes in brain tissue, lead to a deficit of neurological and cognitive functions, which in turn correlate with the severity of brain damage [2]. In this connection, the need to include drugs with psycho- and neuroprotective effects in the therapy of acute cerebrovascular accidents is obvious and beyond doubt [3]. In experiments on rats, the positive effect of pyrimidine derivatives on neurological deficits resulting from impaired cerebral hemodynamics was proved [4], as a result of which it becomes expedient to further study pyrimidine derivatives as agents with neuroprotective effects.

**Objective:** 

To evaluate the effect of the PIR-12 compound on anxiety and motor activity in experimental global ischemia of the rat brain.

# 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, LLC Gedeon Richter Pharma)[6]. The fourth group was administered the pyrimidine derivative PIR-12 (50 mg/kg), synthesized at the department of organic chemistry of the Pyatigorsk Medical and Pharmaceutical Institute (Pyatigorsk, Russia) branch of Volgograd State Medical University[7]. All objects were injected intraperitoneally for ten days before the operation. In the second and subsequent groups global brain ischemia was simulated by bilateral occlusion of the common carotid arteries (under chloral hydrate anesthesia 350 mg/kg) [8, 9]. A day after the reproduction of ischemia, the behavioral activity of the animals was assessed in the "Elevated plus maze" (EPM) test. The Elevated plus maze is a behavioral test to study the activity, emotional state and level of anxiety in laboratory animals [10]. Animal behavior is recorded within 3 minutes. The indicators of anxiety were considered — the time spent on the central site (CS), the time in the open arms of the

maze (OA), the time in the enclosed arms of the maze (EA), the number of grooming acts. Changes in motor activity were judged by indicators — the number of transitions between arms (vertical activity), stance, hanging (horizontal activity). 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

Under conditions of experimentally simulated cerebral ischemia in rats, an increase in the degree of anxiety was observed, which was manifested in a decrease in the time spent on the central site by 74,3% (p<0,05) and the time in the open arms of the maze by 71,2% (p < 0,05), as well as an increase in the time in the enclosed arms of the maze by 55,4% (p<0,05) and the number of grooming acts by 3 times (p<0.05) compared with the initial indicators (CS — 28,4±1,8 sec., OA — 49,7±3,9 sec., EA — 101,9±5,3 sec., Grooming — 3,2±0,5 acts). Relative to the FO group of animals in negative control rats, the time in the center and in the open arms was significantly lower by 73,4% (p<0,05) and 72,3% (p<0,05), the time in the enclosed arms and grooming was higher by 57% (p<0,05) and 2,9 times (p<0,05), respectively. Under conditions of prophylactic administration of Cavinton, the time at the central site by 135,6% (p<0,05) and open sleeves was 93,7% (p<0,05) higher, the time in enclosed arms and grooming acts by 14,7% (p<0,05) and 3,4 times (p<0,05), respectively, were lower in relation to the analogous data of the NC group of animals, which may indicate a decrease in the level of anxiety in individuals. In the setting of intraperitoneal administration of the PIR-12 compound, the time in the center and in the open arms was 3,2 (p<0,05) and 3,1% (p<0,05) times higher, and the presence in the enclosed arms and the number of acts grooming is lower by 28,9% (p<0,05) and 4,1 times (p < 0.05), respectively, compared with rats in the negative control group. In comparison with the group of rats treated with Cavinton, CS and OA in animals that were injected with PIR-12 was statistically significantly higher by 34,3% (p<0,05) and 59,6% (p<0,05), respectively. The time in the enclosed arms of the labyrinth, on the contrary, significantly decreased by 16,6% (p<0,05) in rats receiving PIR-12 relative to the reference drug Cavinton.

In addition to changes in the degree of anxiety in conditions of irreversible ligation of the carotid arteries, there is a decrease in horizontal and vertical motor activity. Thus, in animals of the NC group, a decrease in the number of transitions between the arms of the maze by 74% (p<0,05), hanging by 94% (p<0,05) and stands by 90,9% (p<0,05) compared with indicators before the reproduction of ischemia (transitions –  $7,7\pm0,8$ , hanging —  $8,4\pm1,1$ , stands —  $14,3\pm1,8$ ). In comparison with falsely operated rats, similar indicators in this group were also significantly lower: the number of transitions by 74,4% (p<0,05), hanging - by 94% (p<0.05), stands - by 90.6% (p<0.05). Against the background of taking the reference drug Cavinton, compared with untreated individuals, there was a significant change in the number of hanging (it was 6,6 times higher (p<0,05)) and stands (it was 2,7 times higher (p<0,05) in there were no significant differences in the "number of transitions " indicator. The use of the test substance PIR-12 led to an increase in motor activity, so the number of hanging was 7,4 times higher (p < 0.05), and the number of racks was 4,2 times higher (p<0,05) in relation to untreated rats. There were no statistically significant differences in the indicators characterizing the level of locomotor activity between the groups of rats treated with compound PIR-12 and the reference drug Cavinton.

## CONCLUSION

In the experimentally simulated cerebrovascular insufficiency, the pyrimidine derivative under the laboratory code PIR-12 made it possible to reduce the degree of anxiety and increase the locomotor activity and showed an effect that was superior in strength to the comparison drug Cavinton.

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Group	Time spent in the central square (sec.)	Time in the open arms of the labyrinth (sec.)	Time in the enclosed arms of the labyrinth (sec.)	Grooming (number of acts)			
	M±m						
FO	27,4±1,8	51,7±4,4	100,9±4,1	3,3±0,5			
NC	7,3±1,3#	14,3±1,4#	158,4±1,7#	9,5±1,3#			
Cavinton	17,2±1,9*	27,7±3,8*	135,1±4,6*	2,8±0,5*			
PIR-12	23,1±1,2*/∆	44,2±4,5*/∆	112,7±4,9*/∆	2,3±0,6*/∆			

**Table 1.** Assessment of the changes in the level of anxiety in the "Elevated plus maze" test against the background of the administration of the PIR-12 compound and Cavinton in experimental cerebral ischemia in rats

**Note:** F0 — false-operated rats; NC — negative control rats; Cavinton — a group 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);  $\Delta$  — statistically significant as compared to rats treated with Cavinton (p<0,05).



**Fig. 1.** Assessment of locomotor activity in the Elevated plus maze" test against the background of the administration of the PIR-12 compound and Cavinton in experimental cerebral ischemia in rats

**Note:** F0 — false-operated rats; NC — negative control rats; Cavinton — a group rats treated with Cavinton; PIR-12 — a group of rats treated with PIR-12; # — statistically significant as compared to the F0 rats (p<0,05); \* — statistically significant as compared to the NC rats (p<0,05); $\Delta$  — statistically significant as compared to rats treated with Cavinton (p<0,05).

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# SUBSTANTIATION OF THE COMPOSITION OF A RECTAL DOSAGE FORM CONTAINING KETOPROFEN AND GLUCOSAMINE SULFATE FOR THE TREATMENT OF RHEUMATOID ARTHRITIS

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ABSTRACT — In the course of this study, it was found that currently more than 30 million people are forced to take non-steroidal anti-inflammatory drugs (NSAIDs) daily. It is reliably known that nonsteroidal anti-inflammatory drugs with prolonged use cause a number of side effects, the main of which are erosive and ulcerative changes in the mucous membrane of the gastrointestinal tract. Special attention is paid to the classification and comparative evaluation of various NSAIDs and possible complications in the case of their use. As a result of the performed analysis, it was found that rectal forms have been developed for most NSAIDs, which is convenient for use in pediatric practice, in patients with gastropathies, nausea, vomiting, swallowing disorders, in an unconscious state. The common sides of their pharmacological action are anti-inflammatory, antipyretic, analgesic effects due to inhibition of cyclooxygenase I, II activity and leading to disruption of arachidonic acid metabolism with suppression of prostaglandin synthesis in the focus of inflammation. Data analysis showed that NSAID suppositories are prescribed for: articular syndrome; pain in the spine, neuralgia, myalgia, traumatic inflammation of soft tissues and musculoskeletal system; rheumatism, diffuse connective tissue diseases; dysmenorrhea.

**KEYWORDS** — rectal dosage form, non-steroidal antiinflammatory drugs (NSAIDs), ketoprofen, rheumatoid arthritis, a combination of dexketoprofen and glucosamine hydrochloride.

# INTRODUCTION

Rheumatoid arthritis (RA) is an autoimmune disease of unknown etiology characterized by symmetrical erosive arthritis (synovitis) and a wide range of extra–articular (systemic) manifestations. Rheumatoid arthritis is an extremely common disease affecting approximately 1% of the world's population. The cardinal signs of rheumatoid arthritis include steadily progressive joint damage (chronic pain, deformity, dysfunction), leading to disability and even a decrease in the life expectancy of patients. 4 groups of medicines are used for the treatment of rheumatoid arthritis: 1) nonsteroidal anti-inflammatory drugs (NSAIDs); 2) "basic" drugs; 3) glucocorticosteroids (GCS); 4) "biological" agents.

Nevertheless, NSAIDs induce damage to the gastrointestinal tract throughout its entire length from the esophagus to the rectum.

A more effective dosage form is rectal suppositories with NSAIDs, which is convenient for use in pediatric practice, in patients with gastropathies, nausea, vomiting, swallowing disorders, in an unconscious state.

#### Purpose of study

There is a justification of the composition, technology of a new rectal dosage form containing ketoprofen and glucosamine sulfate, which will reduce the ulcerogenic effect of ketoprofen due to joint use with glucosamine.

# MATERIALS AND METHODS

The article presents an overview of the results of scientific research on the treatment of rheumatoid arthritis with various nonsteroidal anti-inflammatory drugs. The object of the study were preparations of nonsteroidal anti-inflammatory drugs (ketoprofen, ibuprofen, diclofenac, indomethacin, etc.). The search was carried out in electronic databases: PubMed, Cyberleninka, Elibrary Scopus. The search queries were performed in Russian and English.

## RESULTS

Autoimmune rheumatoid arthritis is a progressive disease that affects the joints, leading to chronic pain, deformity, and dysfunction. Ultimately, the disease leads to disability (Fig. 1).

The development and progression of rheumatoid arthritis is determined by a complex combination of genetically determined and acquired defects ("imbal-



ance") of normal (immuno) regulatory mechanisms that limit the pathological activation of the immune system in response to potentially pathogenic, and often physiological stimuli. This leads to a rapid transformation of the physiological (protective) acute inflammatory reaction into chronic progressive inflammation, which is an integral feature of RA.

Nonsteroidal anti-inflammatory drugs prescribed for rheumatoid arthritis, with prolonged use, cause a number of side effects, the main of which are erosive and ulcerative changes in the mucous membrane of the gastrointestinal tract.

Against the background of course admission, acute, usually multiple, gastroduodenal erosions and ulcers occur with predominant localization in the antrum of the stomach.

The danger of gastroduodenal erosions and ulcers, the development of which is caused by taking NSAIDs, is that they are often multiple in nature and are often accompanied by sudden gastrointestinal bleeding. The mechanism of the negative effect of NSAIDs on the gastric mucosa is mainly associated with inhibition of the synthesis of prostaglandins, which have a cytoprotective effect, as well as with a decrease in the production of gastric mucus and a change in its qualitative composition, violation of capillary circulation in the gastric mucosa, epithelial cell regeneration processes, accumulation of free radicals (Table 1).

Medications used in the treatment of arthritis are divided into two groups: fast-acting and slow-acting symptom-modifying. The drugs of the first group are aimed at suppressing pain and normalizing the function of the affected joints. These include nonsteroidal anti-inflammatory drugs and glucocorticoids. The appointment of NSAIDs to patients with arthritis is justified by the persistence of the inflammatory process in the joints and periarticular soft tissues. However, these drugs actively affect the metabolism of cartilage, mainly reducing the synthesis of proteoglycans, type II collagen and hyaluronic acid, and thus contribute to the progression of structural changes. In addition, NSAIDs increase the risk of developing gastropathies and cardiovascular catastrophes, even in people without concomitant pathology.

**Fig. 1.** Development and progression of rheumatoid arthritis Type 1 — Not progressive 5–20% Type 2 — Moderately progressive5–20% Type 3 — Progressive 60–90%

**Table 1.** Relative risk of gastrointestinal complications when using different NSAIDs

NSAIDS	Relative risk
ibuprofen	1.84
naproxen	4.10
diclofenac	3.34
indomethacin	4.14
ketoprofen	3.92
ketorolac	11.5
meloxicam	3.47
piroxicam	7.43
rofecoxib	2.32
nimesulide	3.83

Among the drugs of the second group – symptommodifying drugs of delayed action, the primary role belongs to the natural component of the cartilaginous intercellular substance glucosamine, which is the most studied among the drugs of this group. The drugs of the second group have not only a symptom-modifying effect, but are able to control the course of the disease, slow down the rate of its progression, stabilize structural changes in hyaline cartilage and prevent the development of inflammatory conditions in intact joints. In this regard, they are considered as pathogenetic (basic, chondromodulating) drugs in the treatment of arthritis.

Preparations containing glucosamine are characterized by a slower development of symptom-modifying action, pronounced aftereffect, when after discontinuation of treatment the effect persists for another 4 to 8 weeks.

## CONCLUSION

Therefore, it can be concluded that the presence of side effects, which are inherent in most NSAIDs, necessitate the search for new methods of their use, in which the toxic effect on the body is removed or reduced, and the use of a combination of ketoprofen + glucosamine in rectal dosage form makes it possible to avoid such a negative effect, while having a potential structural-modifying effect.

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# WHAT ANTHROPOMETRIC PARAMETERS AFFECT THE LINEAR DIMENSIONS OF LEFT LIVER LOBE?

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ABSTRACT — The anthropometric parameters and linear ultrasound diameters of left liver lobe were taken in 212 adult healthy volunteers (90 men and 122 women). In men anthropometric parameters correlate with linear diameters of the left liver lobe much more than in women. Anteroposterior diameter of the left liver lobe increasingly correlates with anthropometric parameters both in men and women. The most significant correlations were detected both in men and women between anteroposterior diameter of the left liver lobe and body weight, body mass index and waist circumference.

**KEYWORDS** — anthropometric parameters, ultrasound, linear dimensions of the liver, left liver lobe, gender differences.

# INTRODUCTION

It is well known that left liver lobe may have different forms ranging from practically non-existent to very short and deep or, on the contrary, very long and flat[1, 2, 3]. Thus, one of the anatomical types of left liver lobe's structures is a so called «beaver's tail» («the beaver tailed liver»): such left liver lobe is elongated and wraps around the spleen [4].

Saritha S. etal. consider that knowledge of the liver anatomy in normal and variable forms provides safety during segmental resections of liver. It also helps to avoid errors in interpretation of different methods of diagnostic visualization[3].

Chapligina E.V. etal. [5] has found that anteroposteriordiameter of the left liver lobe is statistically significantly differ in pyknic and asthenic types  $(8,19\pm1,38 \text{ cm} \text{ and } 6,36\pm0,87 \text{ cm}, \text{ respectively}).$ Whereas the left liver lobe craniocaudal diameter doesn't depend on the body type. However, they used abdominal spiral computed tomography for measurement of left liver lobe and said nothing about gender differences. Our previous study has shown[6] that the anteroposterior diameter of left liver lobe in men is larger than in women, but at that study we didn't pay attention to anthropometric parameters. Therefore, it is expediently to identify the relationship between anthropometric parameters and linear diameters of the liver in both genders.

The aim of our study was to determine the correlation between diameters of left liver lobe and anthropometric parameters in men and women, which could be used in clinical practice.

# MATERIALS AND METHODS

We have carried out measurements of anthropometric parameters and ultrasound examination of abdominal cavity's organs with determining of linear dimensions of the left liver lobe in 212 healthy volunteers (90 men 122 women) aged 18 to 69 years.

Height, weight, infrasternal angle, chest circumference (CC) and waist circumference (WC) were measured. The posterior semicircle of CC matches with the line just below the inferior scapula's angle, the anterior semicircle of CC in men crosses the nipples and matches with the line just below mammary gland in women. Waist circumference (WC) was measured at the level of the navel. Thickness of subcutaneous adipose tissue of anterior abdominal wall (SAT of AAW) was measured at 3 cm to the right side from navel with ultrasound linear transducer. Body mass index (BMI) was calculated using standard formula.

Ultrasound examinations of liver were performed with convex transducers using Aixplorer (Supersonic Imagine, France), SonoScapeS6 (China) andMindray-DC-8 (China) ultrasound systems. All patients were investigated in the supine position with the both arms placed above the head, the stretched legs and with quiet breathing.

Leftlobe has been measured either in the median line under xiphisternum or in the right parasternal line due to the anatomical form of left lobe. Transducer was used with no tilt to the right or the left side, so vena cava inferior or aortashould not be visualized on the sonogram. We have measured anteroposterior diameter (AP, depth), craniocaudal diameter (CC, length) and caudate lobe's (CL) thickness (Fig. 1).



**Fig. 1.** Ultrasound measurements of the left liver lobe in a volunteer U., 29 years old. Transducer orientated longitudinally in the midline under xiphisternum. Yellow color indicates the anteroposterior diameter of the left lobe, white — the craniocaudal diameter of the left lobe, blue — the thickness of the caudate lobe

All data were analyzed using the Statistical Package for the Social Sciences 23.0 (SPSS) software recommended for analysis of biomedical data. Correlation was assessed using Spearman's rank correlation coefficient. Chaddock's scale was used for the assessment of correlation [7]. P-values of less than 0.05 were considered statistically significant.

# FINDINGS

The correlation and its statistical significance between anthropometric parameters and linear dimensions of the left liver's lobe in men and women are represented in the Table 1.

We have found that correlation has significant differences between men and women.

A noticeable positive correlation (0,5-0,7) between anteroposterior diameter and such parameters as body weight, BMI, CC and WC and

thickness of SAT of AAW was found in men. A noticeable positive correlation (0,5-0,7) between anteroposterior diameter and such parameters as body weight, BMI and WCwas also found in women. A moderate positive correlation (0,3-0,5) was determined between anteroposterior diameter and such parameters as infrasternal angle, CC and thickness of SAT of AAW in women.

No significant correlation between craniocaudal diameter and antropometric parameters was detected either in men or in women. Moderate positive correlation (0,3-0,5)between craniocaudal diameter with BMI, thickness of SAT of AAW, WC and CC was noticed in men. It is possible to suggest that correlation coefficients between thickness of CL andanthropometric parameters are too minor to be used for practical purposes.

# CONCLUSION

Anthropometric parameters in men are more consistent with the linear diameters of left liver lobe than in women.

Thus, the anteroposterior diameter is the most reproducible ultrasound linear dimension of left liver lobe[8, 9] as well as that it is the one which correlates very good with

Anthropo-metric	AP		СС		CL	
parameters	men (n=90)	women (n=122)	men (n=90)	women (n=122)	men (n=90)	women (n=122)
height	0,132	-0,103	0,259	0,058	0,077	-0,150
	(p = 0,216)	(p = 0,259)	(p = 0,014)	(p = 0,529)	(p = 0,470)	(p = 0,099)
weight	0,617	0,534	-0,160	-0,091	0,270	0,121
	(p < 0,001)	(p < 0,001)	(p = 0,132)	(p = 0,317)	(p = 0,010)	(p = 0,184)
ВМІ	0,643	0,563	-0,407	-0,142	0,272	0,200
	(p < 0,001)	(p < 0,001)	(p < 0,001)	(p = 0,118)	(p = 0,009)	(p = 0,027)
infrasternal angle	0,281	0,417	- 0,113	-0,024	0,096	0,176
	(p = 0,007)	(p <0,001)	(p = 0,288)	(p = 0,795)	(p = 0,369)	(p = 0,053)
СС	0,669	0,438	-0,397	-0,117	0,225	0,101
	(p < 0,001)	(p <0,001)	(p <0,001)	(p = 0,198)	(p = 0,033)	(p = 0,270)
WC	0,674	0,507	-0,345	-0,097	0,302	0,136
	(p < 0,001)	(p < 0,001)	(p = 0,001)	(p = 0,287)	(p = 0,004)	(p = 0,134)
thickness of SAT of AAW	0,518	0,411	-0,446	-0,286	0, 220	0,097
	(p < 0,001)	(p < 0,001)	(p < 0,001)	(p = 0,001)	(p = 0,037)	(p = 0,288)

anthropometric parameters both in men and women. The noticeable positive correlations were detected in men and women between anteroposterior diameter of the left liver lobe and body weight, body mass index and waist circumference. These parameters could be used for assessment of ultrasound liver's dimensions.

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# LIGHT- AND ELECTRON-MICROSCOPIC CHARACTERISTICS OF CHANGES IN THE THYROID GLAND DURING CHRONIC HYPOXIA

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**ABSTRACT** — THE INVESTIGATION AIMS were to study the ultrastructural changes in the cellular and extracellular matrices of the thyroid gland in the norm and chronic hypoxia.

MATERIALS AND METHODS: 40 male white rats were divided into a test and control group. Animals of the control (I) group (n=20) were not subjected to intervention, in the test group (n=20) a model of chronic hypoxia was created the animals were kept in a special ventilation barochamber daily for 2 hours 5 times a week. Anatomical, histological, histochemical, electron microscopic and morphometric methods were used. Morphometric parameters were calculated by use of program Statistica 10 (StatSoft.Inc.) (statistical processing was performed using W — Wilcoxon test (paired samples) with the control group). **RESULTS:** Pathomorphological picture of the thyroid gland of rats subjected to hypobaric hypoxia, on the 15th day of the study was characterized by the acceleration of adaptive processes in gland cells and the development of reparative regeneration. Small structural changes in thyrocytes observed on the 15th day of the experiment show that the gland cells are not completely restored, adaptive processes do not occur completely. The area  $(12.21\pm1.43)$  and the diameter  $(6.25\pm0.48)$  of the nuclei are relatively increased compared to the control group.

Analysis of samples from the thyroid gland of animals adapted to chronic hypoxia on the 30th day of the study shows that the gland cells adapted to the new conditions and completely restored their normal structure and size. The morphometric parameters of the thyroid gland were relatively close to the norm (normal cell diameter  $(5.96\pm0.38)$  and the area of the nuclei  $(12.11\pm1.43)$ ). CONCLUSION: The results of the studies showed that under the influence of prolonged hyperbaric hypoxia, the body's resistance to it, especially the thyroid gland, increases, and it adapts to hypoxic conditions, and this is accompanied by the processes of structural reorganization of organs. Thyrocytes of the thyroid gland adapt earlier to chronic hypoxia and respond to this by hyperplasia and hypertrophy and differentiation of thyrocytes.

**KEYWORDS** — thyroid gland, chronic hypoxia, thyrocytes, follicles.

### INTRODUCTION

Hypoxia is a process depending on the degree and duration of hypoxic effect, leading to alterative changes in cells, especially to a complex of metabolic disorders, in which energy processes play a key role. Hypoxia develops in conditions of oxygen deficiency in the environment, as well as as a result of various pathologies associated with impaired oxygen transport function of the blood. Oxygen deficiency, which causes structural, functional and metabolic insufficiency of cells, in all its manifestations ultimately leads to a decrease in the supply of oxygen to the tissues. And this leads to the appearance of morphofunctional changes in the organs of not only the respiratory, nervous and cardiovascular systems but also the endocrine system, especially in the thyroid gland [1-3]. At the same time, the thyroid gland plays a key role in regulating the functions of various organs and systems in the processes of vital activity of the body, as well as in the formation of adaptive reactions against adverse environmental factors [4–6].

Some scientists note that the hypoxic condition caused by intensive physical work, as well as appearing in high-altitude conditions, plays an important role in the occurrence of some morphological changes and thyroid dysfunction [7-9]. The authors show that the state of periodic hypoxia, which occurs to one degree or another, is a common phenomenon for many forms of labor, sports and military activities [10-14].

Despite all the studies of pathomorphological changes in the thyroid gland, as the main mechanism of adaptation processes in the body during hypoxia of various origins, the analysis of the literature data gives us reason to re-conduct a broad comparative analysis of these changes in the formation of adaptation mechanisms. *The objective of this study* 

was to investigate the features of the ultrastructural organization of cellular and extracellular components of the thyroid gland in normal and chronic hypobaric hypoxia.

# MATERIALS AND METHODS

Materials of the study were 40 healthy white male rats weighing 180–200 grams, divided into 2

groups. Animals of the control group (n=20) were not subjected to intervention, animals included in the hypoxia group (Group II) (n=20) were subdivided into 2 equal subgroups (subgroup 1 and subgroup 2) with the duration of the experiment for 15 and 30 days accordingly and were kept in a special barochamber with a temperature of 19–20° C, atmospheric pressure equal to the pressure at an altitude of 2000–3000 m above sea level, 5 times a week for 2 hours every day. Particles of natron lime (Ca(OH)<sub>2</sub> 81%+NaOH 3,4%+H<sub>2</sub>O 15,6%) were applied to absorb CO<sub>2</sub>. All experiments were carried out within 10:00–15:00 hours, which is considered the lightest time of the day.

By the  $15^{\text{th}}$  and  $30^{\text{th}}$  days of the experiment, the rats of both groups (groups I and II) were euthanized by decapitation, accordingly. At the same time, all painful procedures were performed under surface ether anesthesia and general anesthesia using a 2–2,5% solution of thiopental sodium (100 mg/kg) injected into the peritoneal cavity of animals. After decapitation of the animals, the thyroid gland was completely extracted, materials were taken for anatomical, histological, histochemical, electron microscopic and morphometric studies.

The preparations were first macroscopically evaluated, and then the pieces of the thyroid gland were fixed with a 2,5% solution of glutaraldehyde in a 0,1 M phosphate buffer (pH 7.4) and 2% paraformaldehyde, post-fixed (2 hours) in a solution of 1% osmic acid made in the same buffer, dehydrated, respectively, in solutions of 50°, 70°, 80°, 90° ethyl alcohol (30 minutes), in solutions of 96° and 100° ethyl alcohol (1 hour) and blocks of Araldite-epon and spur resins were made from them [15].

Semithin (1–2 µm) and ultrathin (70–100 nm) sections (by LKB-III, Leica EM UC7 ultratoms) were made from the obtained blocks, which were colored in various ways (methylene blue with fuchsin, uraniumacetate and pure lead citrate). Semithin section viewed under the light microscope «Olympus BX-41». Ultrathin sections 70–100 nm thick were stained with 2% uranium-acetate solution and 0,6% pure lead-citrate and studied under JEM-1400 transmission electron microscope (JEOL, Japan). Morphometric parameters were analyzed statistically using Statistica 10 (StatSoft Inc., USA) software and the Mann-Whitney U-test was performed.

The animal research was carried out in the Department of Pharmacology, Experimental Surgery and the Electron Microscopy Laboratory of the Scientific Research Center of AMU based on ethical principles approved by the Commission on Ethics Rules under the Ministry of Health of the Republic of Azerbaijan on 21.04.2008 (Protocol No. 31) and used following the requirements of the Bioethics Committee.

# RESULTS

Macroscopically, the thyroid gland is clearly distinguished in front of the trachea under the larynx. In histological preparations, the connective tissue capsule covering the thyroid gland, thin trabeculae extending from the capsules into the thickness of the gland, as well as the lobules of the organ separated from each other by trabeculae are visible.

In histological preparations, small follicles consisting of cylindrical cells predominate in the central part of the gland, and large follicles consisting of cuboidal cells predominate in the peripheral part compared to the central part. A colloid in the form of a viscous liquid evenly fills the lumen of the follicles. The cytoplasm of thyrocytes stained with methylene blue with fuchsin and round nuclei, the well-developed interlobular, interfollicular connective tissue of the thyroid gland and blood capillaries are well visualized.

According to electron microscopic studies in the thyroid gland of rats included in the control group, thyrocytes are located in one layer on the basement membrane. There is a close connection between follicular thyrocytes through well-developed numerous lamellae. Round nuclei are located in the center of cells, the membrane of the nucleus and the nucleolus are well identified. Microvilli, on the apical surface of thyrocytes facing the follicle lumen, are observed.

The study of ultrathin sections showed that the basal surface of thyrocytes is folded, which increases the contact of thyrocytes with the perifollicular cavities. On the lateral surfaces of thyrocytes, finger-like protrusions entering into the corresponding indentations of the lateral surfaces of neighboring cells are well distinguished, Thyrocytes have well-developed organelles-ribosomes, mitochondria, lysosomes, endoplasmic reticulum and Golgi complex. A few ribosomes and lysosomes are unevenly distributed in the cytoplasm and are mainly located around the Golgi complex (Fig. 1).

The pathomorphological picture of the thyroid gland of rats subjected to hypobaric hypoxia by the 15th day of the experiment is characterized by an acceleration of adaptive processes in thyrocytes and the development of regeneration.

Under chronic exposure to hypoxia, the structure of the thyroid gland did not undergo significant changes. The capsule of the gland is thickened, but not deformed, although the boundary between the central and peripheral parts of the gland is not clear.

Mild structural changes in thyrocytes observed by the 15<sup>th</sup> day of the experiment show that the thyrocytes are not completely restored, the adaptation processes are slow. Microscopically, the cytoplasm is weakly oxyphilic, foamy in some cells, pale stained with hematoxylin-



**Fig. 1.** TEM. Control group: the structure of the thyroid gland. Stain: uranium-acetate and pure lead citrate. Scale:  $2 \mu m$ 

eosin. Although the morphological structure of the stroma of the gland corresponds to the norm, the destruction of collagen and reticulin fibers is detected, signs of local metachromasia are noted. Despite perivascular and pericellular edema, diapedesis hemorrhage and hyperemic foci are not observed during microscopic examination. Capillaries also do not differ from ordinary capillaries in diameter and structure of the walls. But in some histological preparations, the diameter of the capillaries is wide and the contours are fuzzy.

By the 15<sup>th</sup> day of the experiment, the electronograms clearly show that a small amount of lipid droplets is found in the cytoplasm of thyroid cells, and glycogen grains are not detected due to hypoxia. During the regeneration of the central part of the gland, hypertrophy, and hyperplasia of cell organelles are observed.

The contours of the cytoplasm and nuclei are clear, the nucleus is located in the center of the cell, and the lysosomes, ribosomes, endoplasmic network, and Golgi complex are located around it. The area of the nuclei  $(12.21\pm1.43)$  and the diameter  $(6.25\pm0.48)$  is relatively increased in comparison with the control group animals (Table 1).

There is paleness of the nuclear chromatin of thyrocytes in the peripheral zone, a relative thinning of the nuclear and basement membranes, and weak mitochondrial hyperplasia, clear boundaries of the basement membrane, and complete cell recovery in epithelial cells. Although the same analogous changes are found in the thyrocytes of the central zone, the morphologically cuboidal epithelium becomes rarer under the influence of hypoxia, and a small number of lipid droplets in the cytoplasm is also observed.

Stratification in the basement membranes of the capillaries, vascularizing follicles, hypertrophy of endothelial cells, and narrowing of the lumen of capillaries are revealed. In addition, due to hypoxia, there is an increase in the number of fibroblasts around the capillaries, filling the interstitial substance with collagen and fibrous tissue. This, in turn, leads to a narrowing of the capillary lumen and a deepening of hypoxia.

The analysis of samples taken from the thyroid gland of animals adapted to chronic hypoxia by the 30<sup>th</sup> day of the experiment shows that the gland cells adapted to the new conditions and completely restored their normal structure and size, there was a restructuring of the parenchyma and stroma of the gland tissues.

Macroscopically, the thyroid gland differs little from the thyroid gland of the control group animals. The capsule covering the glands, lobules, septa separating them, as well as the boundaries between the central and peripheral zones of the gland are visualized (Fig. 2). Although the structure of the thyroid gland in histological preparations taken from animals on the last day of chronic hypoxia is close to the norm, it is possible to see the development of fatty and hydropic degeneration in some cells after hypoxia. But these changes are focally observed in some cells.

The follicles and thyrocytes of the gland have completely restored the structure as a result of proliferation and differentiation, the colloid is transparent and dense, evenly fills the lumen of the follicles. On histological preparations, attention is drawn to the develop-

*Table 1.* Morphometric parameters of thyroid cells in the norm and chronic hypoxia ( $M \pm m$ ), (min-max)

	Thyroid gland								
	Diameter of cells		Diameter of nuclei			Area of nuclei			
	Duration of the experiment N (days)		N	Duration of the experiment (days)		N	Duration of the experiment (days)		
		15 <sup>th</sup> day	30 <sup>th</sup> day		15 <sup>th</sup> day	30th day		15 <sup>th</sup> day	30 <sup>th</sup> day
Ν	10	10	10	10	10	10	10	10	10
MM±m	5,94±0,38	6,06±0,37	5,96±0,38	6,04±0,49	6,25±0,48	6,09±0,48	12,08±1,43	12,21±1,43	12,11±1,43
M <sub>min-max</sub>	3,95-7,67	4,12-7,79	3,98-7,69	3,59-7,92	3,73-8,13	3,61-7,97	3,91-20,01	4,02-20,12	3,97-20,03

\* *Note:* N — control group; n — number of animals;  $M \pm m$ : M — the average variation, m — standard error.



*Fig. 2.* Semi-thin section. The structure of the thyroid gland on the 30<sup>th</sup> day of hypoxia. Stain: methylene blue with fuchsin. Magnification: ×40

ment of fibrous tissue among the follicles, an increase in the number of fibroblasts, as well as capillaries.

Electron microscopically, hyperplasia of cytoplasmic organelles, thickening of the plasmalemma and basement membrane, their boundaries, as well as the contours of nuclei and nucleoli located in the center of cells and nuclear chromatin can be observed. Noticeable microvilli on the apical surface of thyrocytes, and protrusions on the lateral surfaces reflect the complete restoration of thyrocytes. The normal cell diameter  $(5.96\pm0.38)$  and the area of the nuclei  $(12.11\pm1.43)$ , as well as the noticeable restoration of cristae in the mitochondria, once again shows the supply of oxygen to the cells (Table 1). The narrowing of the capillary lumen attracts attention, but the structure of all three layers of the basement membrane of the capillary loops is close to normal histological structures (Fig. 3). By the 30<sup>th</sup> day of the experiment, the morphometric parameters of the thyroid gland were also relatively close to the norm.

Thus, the results of studies have shown that as a result of short-term and long-term exposure to hypo-



**Fig. 3.** TEM. 30<sup>th</sup> day of hypoxia. Stain: uranium-acetate and pure lead citrate. Scale: 2 µm

baric hypoxia, various morphological changes occur in the cells of the thyroid gland. So, if the analysis of histological and ultra-thin sections at an early stage of the experiment showed the effect of acute hypoxia, then in the cells of the thyroid gland, as well as in the intercellular spaces, there is the formation of edema, stagnation, as well as cell destruction, but at the end of the study there is a recovery process at a noticeable level, there is a process of restoration of follicles, thyrocytes, cytoplasmic organelles and an increase in their size and number. The manifestation of this result at the ultrastructural level can be assessed as the restoration and adaptation of thyroid cells to hypoxic conditions.

## DISSCUSSION

Under the influence of hypoxia, especially chronic hypoxia, the reactivity and adaptation tension of the pituitary-thyroid, pituitary-adrenal and simpato-adrenal systems increases. As a result of hypoxia, the signs of follicular destruction in the thyroid gland tissue are clearly noticeable [2]. During the experiment, the presence of resorption vacuoles in the colloid, desquamation of some thyrocytes into the follicular cavity and changes in the ratio of follicle-colloid relationship were noted [16]. According to most authors, the process of reconstruction of the thyroid gland takes place at this time. Due to these processes, lymphatic drainage weakens, as a result of which edema develops and the proportion of interstitial tissue increases [8]. Thus, the authors concluded that the sanogenic level of hypoxia can increase the functional activity and the processes of physiologic regeneration of the thyroid gland in adult animals [4, 6, 16]. According to the results of our study, under the influence of chronic hypoxia, significant structural changes occur in the cells of the thyroid gland, and these changes result in the reconstruction of the structure of the gland. Our results once again confirm the views of these authors.

# CONCLUSION

To sum up it should be noted that the thyroid gland plays an important role in the early adaptation of the body to hypoxia. With prolonged hypoxia, the body's resistance to it increases, mainly the thyroid gland adapts to hypoxic conditions, which is accompanied by the processes of structural restructuring of organs. On the other hand, it should be noted that adaptive changes occur not only depending on the type, nature, duration, and intensity of hypoxia but also morphofunctional features of organs. The structure of the thyroid gland adapts first of all to prolonged hypoxia and responds to this with hyperplasia and hypertrophy and differentiation of thyrocytes. The manifestation of this result at the ultrastructural level, as we noted above, maybe due to the adaptation of thyroid tissues to new conditions.

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# EFFECT OF CORONAVIRUS DISEASE (COVID-19) ON HEMOSTASIS SYSTEM IN PATIENTS WITH ACUTE CEREBROVASCULAR ACCIDENTS

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ABSTRACT — Acute viral respiratory infections add to the progression risk of the already existing pathologies, including those of cardiovascular origin. Life-threatening complications emerging against SARS-CoV-2 (severe acute respiratory syndrome), which causes COVID-19 (Coronavirus disease 2019), explain the need to study the cardiovascular effects of COVID-19 in order to offer rational medical care to patients belonging to various age categories. This article presents a comprehensive assessment of changes affecting the main hemostasis parameters in patients with acute cerebral circulatory disorders against the SARS-CoV-2 virus infection. There is special attention paid to the pathophysiological features occurring against the infectious process involving the hemostasis system. Our data shows that the differences in the main hemostatic parameters in patients with acute cerebral circulatory disorders occurring against the SARS-CoV-2 virus infection are due to the degree of the body responsiveness and the severity of the comorbid pathology. Reducing the risk of adverse conditions developing in this category of patients is possible in case of responsible monitoring focusing on the main indicators of the cardiovascular system status, as well as provided there are advanced approaches introduced in order to prevent and treat thrombotic/thromboembolic complications.

**KEYWORDS** — coronavirus, cardiovascular diseases, coronavirus infection 2019, SARS-CoV-2, COVID-19, hemostasis, acute cerebrovascular accident.

## INTRODUCTION

Respiratory viral diseases contribute to the progression of pre-existing chronic non-infectious pathology, cardiovascular diseases (CVD) first of all, while the initially existing CVD associated with other concomitant pathology enhance the likelihood of the infection development and progression. The COVID-19 pandemic caused by a new strain of coronavirus SARS-CoV-2, has led to a rapid increase in the number of cases and high mortality globally. Despite the tropism of SARS-CoV-2 to the lungs, COVID-19 contains a high risk of developing multiple organ failure, including due to damage affecting the cardiovascular system [6, 8, 12]. More than half of all patients with SARS CoV-2 feature multimorbidity, the frequency of that increasing up to 72% in severe COVID-19 cases [5,15].

Under the COVID-19 pandemic, patients with CVD are at risk of severe infection, which is due to high metabolic needs experienced by this category of patients, the needs exceeding the available cardiac reserve, thus creating grounds for the previously existing pathology progression. During the acute period of viral infection, there is a release of pro-inflammatory cytokines occurring (cytokine storm). A sharp increase in the interleukin-6 level in the vascular bed promotes the disturbance in the vasoregulatory function of the microcircular bed vascular endothelium, as well as facilitates thrombosis, microcirculation disorders in organs and tissues, acute myocardial damage, arterial hypertension, heart failure, and the development of multisystemic disorders. Given the background of high virus-induced inflammation, the existing atherosclerotic plaques get destabilized. Proinflammatory cytokines produce a systemic procoagulant and thrombogenic effect, whereas the likelihood of developing thrombotic and thromboembolic complications increases sharply [4, 11, 14].

Cerebral vascular pathology is an extremely serious medical and social issue faced by the society nowadays, which can be accounted for by its wide prevalence (1–4 cases per 1,000 population annually), high mortality rate (up to 35% in the acute period of stroke), significant rat of disability and loss of capacity. The WHO claims that stroke, whose rates are very high among cerebrovascular pathologies, is a global epidemic threatening the life and well-being of the world's population. The annual number of brain strokes registered globally exceeds 7 million, while every fourth case is fatal (WHO, 2018). Research literature contains extensive data concerning hemorheological changes in case of COVID-19, with respective data available regarding an increasing risk of developing acute cerebrovascular accidents (CVA) against the background of COVID-19 [9,13].

A number of researchers stress that the most important pathophysiological link involved in the chain of stroke development is a disturbance in the vascularplatelet hemostasis system, thrombocytes (TrC) playing a key role in this case. In case of a pathology, there are changes to be observed, which affect morphometric parameters of platelets like the average diameter, the area, the shape factor, the polarization, the specific optical density in three spectral ranges (blue, green, red) and the proportion of blue and red [1, 3]. The available literature sources offer scarce details on TrC morphometry in case of a CVA. This issue requires indepth consideration, since the information obtained may allow timely diagnosing and assessing the prognosis for stroke course and outcome.

#### Aim of study:

to study the morphofunctional and geometric parameters of thrombocytes, taking into account the hemostatic parameters of blood plasma in patients with acute cerebrovascular issues in different age groups, in view of the SARS-CoV-2 virus infection background.

## MATERIALS AND METHODS

In order to study changes affecting morphofunctional parameters (number, average volume, anisocytosis index, area, average diameter, shape factor, cell polarization) of thrombocytes, which are due to CVAs, 81 patients with a reliably set diagnosis were examined, undergoing inpatient specialized treatment. The CVA diagnosis was verified based on the clinical presentation and magnetic resonance imaging (MRI) data. During the MRI, the nature of the stroke (ischemic or hemorrhagic), the magnitude and prevalence of focal brain changes were identified. The participants, after signing respective voluntary informed consents, were divided into groups based on their age: Group I — 18 patients (aged 35–49; median age - 42±7); Group II -27 patients (aged 50–60 years; median age  $-55\pm5$ ); Group III — 36 patients (aged 61–75; median age —  $68\pm7$ ). Subject to the aim of the study, the following methods were applied: physical & chemical, conductometric (impedance), mathematical, TrC computer morphometry, coagulation-base. The thrombocyte link indicators were studied based on three features.

The study was carried out on an ADVIA 2120i (Siemens Healthcare Diagnostics) automatic hematology analyzer, with whole blood used as the biological material. Blood sampling was performed in the morning, on an empty stomach. Blood was placed in dry vacuum tubes containing EDTA as an anticoagulant agent. Also, a smear was prepared from the blood for scanning probe microscopy and computer cytomorphometry of thrombocytes. Blood preparations were made by a standard unified method. The study of the hemostasis system was performed on a Sysmex CS-2100i (Sysmex) automatic coagulometer with the following indicators identified: activated partial thromboplastin time (APTT), international normalized ratio (INR), prothrombin index (PTI), prothrombin time (PTT), fibrinogen, antithrombin III, D-dimer. Aggregational examination of thrombocytes is a source offering the most important information for detecting functional disorders of thrombocytes. Aggregation can be primary, which is caused by an inducer, and secondary - due to biologically active compounds released by thrombocytes.

The study was conducted on the BIOLA ALAT-2 220LA (Russia) aggregometer. In our study, we identified the indicators of TrC aggregation with various inducers (ATP, ristocetin, collagen). Morphometric studies of thrombocytes were carried out using the MEKOS-C3 hardware and software complex, as well as the INTEGRA atomic force microscope. The following indicators were identified: the thrombocyte area, the thrombocyte average diameter, the thrombocyte shape factor, the thrombocyte polarization, the blue color fraction, the red color fraction, and the specific optical density (Fig. 1, 2).

The statistical data processing was done with the Microsoft Excel 2013 software as well as employing the package of the SPSS Statistics software (version 22). The critical level of a possible null statistical hypothesis was taken as equal to 0.05.

#### **RESULTS AND DISCUSSION**

The study resulted in significant differences revealed in terms of coagulogram and TrC aggregation in the groups. There was a significant decrease in APTT obtained in Groups II  $(31.6\pm3.6)$  (P<0.02) and III  $(30.4\pm3.6)$  (P<0.02) compared to Group I  $(40.1\pm2)$ . PTV is a parameter, which characterizes the activity of the main blood clotting factors (I, II, V, VII, X), whereas its increase may point at the activation of the hemostasis system, which confirms the results obtained here. There was, for instance, a significant increase in the PTV parameter observed in Group III  $(18.6\pm2.4)$  compared to Group I  $(12.3\pm0.8)$  (P<0.01) and Group II  $(13.9\pm1.1)$  (P<0.05). A change in the entire body of these indicators may be indicative of hypercoagulation that develops in case of a CVA in an older age group within the second maturity period.





Fig. 1. Thrombocytes of a patient (51 y.o.) with CVA and<br/>with the SARS-CoV-2 virus infection in history (MEKOS-<br/>CVA and<br/>C3 hardware and software complex)Fig. 2.CVA and<br/>C3 hardware and software complex)CVA and<br/>(MEKOS-<br/>CVA and<br/>Mote: the arrow points at a giant thrombocyte to be<br/>seen on the background of altered erythrocytes.Fig. 2.



The prothrombin index (PTI) is a laboratory indicator used to describe the external pathway of blood clotting. When increased, it may point at higher clotting, which is of primary importance in the development of a CVA. Our study revealed a significant increase in the PTI in Group III (121.7±2.4) (P<0.01) compared to Group I (90.2 $\pm$ 12.3), with a statistically insignificant PTI increase in Group II (95.4±24.5). There was a significant decrease in the AT-III parameter in Group III (78.3 $\pm$ 6.8) (P<0.05) compared to Group I (93.2±3.6). Besides, a statistically insignificant decrease in this indicator in Group II (89.9±11.5) was observed, too. Antithrombin III (AT-III) is a blood clotting inhibitor. A decrease in its level in case of a developing CVA may indicate a decrease in the anticoagulant system activity, which is confirmed by the results of our studies. The study of such a coagulogram indicator as fibrinogen revealed its highly significant increase in Group III (6.1±0.5) if compared to Group I  $(2.9\pm0.4)$  (P<0.001) and Group II (4.6±0.6) (P<0.05), as well as an increase of this indicator in Group II if matched against Group I (P<0.01). Fibrinogen is not only a blood clotting factor, yet also an acute phase protein, which means its increase may point at not only the development of hypercoagulation, but inflammation, too, as well as tissue damage in case of a CVA.

CVAs are characterized by thrombotic conditions associated with the blood containing substances featuring procoagulant properties, D-dimer in particular (Berkovsky, A.V., 2011). In our study, there was a significant increase of D-dimer observed in Group III ( $9.4\pm0.8$ ) compared to Group I ( $0.25\pm0.2$ ) (P<0.001) and Group II ( $4.6\pm2.6$ ) (P<0.05). There was also a significant increase in this indicator registered in Group II compared to Group I (P<0.05). The obtained data may confirm the presence of a hypercoagulation condition developing in case of a CVA.

The indicators of induced aggregation typically feature a significant increase in aggregation with ristocetin in Groups II ( $138.1 \pm 41.7$ ) (P<0.05) and III (160.7±26.5) (P<0.001) if compared to Group I  $(68.1\pm6.2)$ . As for aggregation with ADP, there no were statistically significant values revealed, yet a tendency towards its increase detected in Groups II (79.8±13.4) and III (87±9.7) compared to Group I  $(71.2\pm10.1)$ . The aggregation with collagen index revealed a significant increase in Group III  $(96.1\pm8)$ (P<0.05) and a statistically insignificant increase in Group II (80.2±13.5) compared to Group I  $(72.5\pm10.6)$ . Therefore, there are changes in the hemostasis system indicators, both in case of CVAs with no concomitant pathology, and in case of CVAs on the background of smoking and SARS-CoV-2. A decrease in APTT, AT-III, an increase in PTI, PTV, fibrinogen, D-dimer, for instance, may point at the development of hypercoagulation with anticoagulation system inhibition. Changes in these indicators point at a strain in the hemostasis system in case of the pathology in question, which also is confirmed by an increase in TrC induced aggregation.

To complete the objectives set within the study, we analyzed the quantitative and morphometric indicators of TrC. In order to identify various changes in the thrombocyte link of hemostasis, the examination implied a statistical analysis of the obtained data. Based on the data, there were some quantitative and morphometric differences of TrC identified in the groups (Fig. 3).

The study showed that the TrC number featured a statistically significant increase in Group II  $(361.4\pm26.6)$  (p<0.01), with a decrease in Group III (190.0±30.0) (P>0.001) if compared to Groups I and II. Unlike the TrC numbers, the PDW and MPV indicators revealed no statistically significant, reliable differences, yet a tendency towards an increase in these indicators was identified in Groups II (PDW 42.3±3.6; MPV 10.2±0.7) and III (PDW 43±6.6; MPV 10.6±0.6). It suggests that these changes in thrombocytes are associated with their activation through a CVA development, namely, an increase in the MPV parameter was noted, which points at the appearance of younger forms of thrombocytes. An increase in the PDW parameter indicates an increase in the TrC anisocytosis. A decrease in the TrC number in case of a CVA against SARS-CoV-2 in different age groups may indicate the severity of the disease course. Since SARS-CoV affects the lung tissue, which may be the site of TrC release from mature megakaryocytes, disturbances in the capillary pulmonary blood flow may be associated with higher consumption and reduced production of thrombocytes (Fig. 4).

The TrC average diameter in Group III (4.5±0.6) (p<0.02) was significantly above that in Groups I  $(2.6\pm0.6)$  and II  $(3.2\pm0.23)$  (p<0.02). There was a significant increase noted in the TrC area in Group III  $(7.8\pm0.5)$  (p <0.02) if compared to Groups I  $(4.8\pm0.27)$  and II  $(5.3\pm0.31)$  (p<0.02). The shape factor describes the degree of the cell edge indentation, which is due to the development of pseudopodia, occurring both with in case of CVAs without concomitant pathologies, and with CVAs on the background of COVID-19. The study revealed a significant increase in the TrC form factor in Group III  $(18.3\pm1.8)$ (p<0.05) compared to Groups I  $(13.9 \pm 2.39)$  and II (14.9±0.81). There were no statistically significant differences detected in the TrC polarization index, however, a tendency towards its increase was determined in Group III  $(0.24\pm0.02)$  compared to Groups I  $(0.2\pm0.07)$  and II  $(0.22\pm0.02)$ . Changes in these TrC geometric parameters are indicative of a hyperactivated thrombocyte link in case of a CVA.

3D scanning probe microscopy and scanning electron microscopy was used to make images of activated thrombocytes in patients with acute cerebrovascular accident on the background of SARS-CoV-2 (Fig. 5–7).

We have revealed that in case of a CVA on the background of the SARS-CoV-2 virus infection in history, there are changes affecting the hemostasis main parameters, as well as quantitative and morphometric



*Fig.3.* Number of TrC in groups: Group I (age — 35–49); Group II (age — 50–60); Group III (age — 61–75)

**Note 1:** P1 — reliability of differences between indicators in Groups I and II; P2 — reliability of differences between indicators in Groups I and III; P3 — reliability of differences between indicators in Groups II and III. **Note 2:** \* — P < 0.05, \*\* — P < 0.01, \*\*\* — P < 0.001.





Fig.4. Average TrC diameter and area in studied groups

indicators of thrombocytes, while the dynamics of changes in the indicators is multidirectional. As far as the hemostasis indicators are concerned, the following was observed: a shortened APTT, an increase in the PTV, PTI, fibrinogen, D-dimer, induced aggregation with various inducers (ADP, ristocetin, collagen), while the severity of the changes was the biggest in Group III. These changes indicate the development of



*Fig.5* (a-d). Activated thrombocyte — 3D projection (size 12 × 12  $\mu$ m). Blood preparation; patient 51 y.o. with a CVA on the background of the SARS-CoV-2 virus infection in history



**Fig. 6.** Activated thrombocyte: a — plane projection (size  $12 \times 12 \mu m$ ); b — scanning electron microscopy (increase  $\times 17500$ ). Blood preparation; patient 51 y.o. with a CVA on the background of the SARS-CoV-2 virus infection in history

hypercoagulation, and a decrease in the anticoagulant system function in case of a CVA. Blood coagulopathic potential occurs through substances entering the bloodstream, where the substances in question gave procoagulant properties, while the sources of these substances are to be found in the decomposition foci of the brain tissue.

It is important to note that the SARS-CoV-2 virus infection is followed with more profound

changes in hemostasis. The obtained results concerning the hemostasis status in the patients are consistent with published research data on the pathophysiological mechanisms behind the development of cardiovascular complications in case of COVID-19: a direct damaging effect of the SARS-CoV-2 virus on pericytes, cardiomyocytes and fibroblasts; an indirect effect that the SARS-CoV-2 virus has on the myocardium under a cytokine storm; a direct damaging effect 60



*Fig. 7. A* — Thrombocyte conglomerates (aggregates), 3D projection (size  $25 \times 25 \mu$ m); B — plane projection (size  $25 \times 25 \mu$ m); C — scanning electron microscopy (increase ×10 000). Blood preparation; patient 51 y.o. with a CVA on the background of the SARS-CoV-2 virus infection in history

of the SARS-CoV-2 virus on the vascular endothelium, resulting in its dysfunction; hypercoagulation due to endothelial dysfunction, increased thrombocyte activity and decreased plasminogen production; pronounced hypoxemia, which leads to increased anaerobic processes, intracellular acidosis and oxidative stress; imbalance between myocardial oxygen demand and its delivery on the background of virus-induced inflammation, hypoxia, oxidative stress, endothelial damage and hypercoagulation; activation of the sympathetic system with stress-induced release of catecholamines into the blood; electrolyte imbalance, which facilitates the development of tachyarrhythmia [2, 7, 10].

## CONCLUSION

1. Diagnostically significant changes in the hemostasis system in patients with CVAs against the background of the SARS-CoV-2 virus infection include increased blood levels of D-dimer, increased prothrombin time, thrombin and activated partial thromboplastin time (APTT). The early stages of the disease feature an increase in the fibrinogen concentration; however, as the disorders progress, the blood levels of fibrinogen and antithrombin decrease. Thrombocytopenia is also associated with the severity and the prognosis of the disease, yet is rarely significant.

2. Out of the examined hemostatic parameters, D-dimer is the most interesting as a marker of severity and unfavorable prognosis in case of a CVA against the background of the SARS-CoV-2 virus infection, since its definition is widely available and standardized. Prothrombin time is of prognostic value, too, however, during hospitalization, its changes in patients with unfavorable prognosis are not as obvious as in case of D-dimer, and will not typically go much beyond the upper normal limit.

3. Patients with a CVA on the background of the SARS-CoV-2 virus infection, when hospitalized, are

recommended to have the blood level of D-dimer, prothrombin time, fibrinogen concentration, identified, as well as to take a detailed general blood test, including such as to detect the level of thrombocytes, followed by regular monitoring of these indicators. These hemostasis indicators, if exceeding the normal values significantly, serve signs of the disease taking a worse turn with the development of serious consumption coagulopathy, where intensification of COVID-19 treatment and/ or the introduction of blood components may be required.

4. The prothrombin ratio and prothrombin time are not recommended to be replaced with the international normalized ratio, since it does not detect relatively small changes that may occur in case of COVID-19.

5. The difference in the main morphometric, hemostatic parameters is a distinctive feature of patients with CVAs on the background of the SARS-CoV-2 virus infection, whereas the variability of these parameters reflects the degree of the body responsiveness, the severity of the disease, as well as it allows making forecast regarding the outcome.

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# HYPOXIC BRAIN DAMAGE IN PREMATURE INFANTS (MORPHOLOGICAL STUDY)

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## INTRODUCTION

Perinatal brain damage in newborns is one of the leading causes of morbidity and mortality, and even in a surviving infant it can result in severe motor and cognitive impairment, as well as disability in various life periods [1–5]. Perinatal hypoxia, especially in premature infants, plays a leading role in these conditions [6, 7, 8, 9]. Severely premature infants born at 22-32weeks' gestation are at high risk of brain damage, leading to death or various central nervous system (CNS) abnormalities. Morphogenesis and angiogenesis are active during these gestational periods, and brain regions have unique anatomical characteristics corresponding to a particular gestational age [10, 11, 12]. Premature birth can dramatically change the trajectory of brain development. Premature infants born before 27 weeks of gestation demonstrate a massive decrease in cortical and subcortical volumes, brainstem volume reduction, an increase in cerebrospinal fluid volume, and loss of cortical gray matter [13]. Ball G. et al. found that severely premature infants have reduced volumes of the thalamus, hippocampus, orbitofrontal lobe, posterior cingulate cortex, and impaired development of such brain areas as the thalamocortical system [14]. Any adverse exposure can disrupt physiological organ development, cerebral circulation, and vascular system integrity. Low cerebral blood flow and fluctuations in systemic blood flow combined with impaired cerebral autoregulation can cause damage to anatomical brain structures accompanied by hemorrhages in premature infants [12, 15].

The microcirculatory system is responsible for the transport of oxygen and nutrients, which aids the physiological development of the brain structures. However, in a premature infant born at 30–33 weeks' gestation,

**ABSTRACT** — THE AIM OF THE STUDY was to investigate the morphological signs of hypoxic damage to the central nervous system (CNS) in premature infants. MATERIAL AND METHODS: The autopsy reports of 20 premature infants were analyzed. The mean gestational age was 25.9±3.7 weeks, median birth weight was 650 [535;940] grams, and height was 32.6±5.4 cm. We analyzed clinical assessment of the infant's condition at birth, the course of the neonatal period, blood gases and acid-base status, the mode and parameters of mechanical lung ventilation, as well as the oxygenation index (OI) and oxygen saturation index (OSI). Macroscopic and histological signs of damage to various brain structures were studied during pathological anatomical examination. Cortex, parietal subcortex, hippocampus, striatum, cerebellum, foci of hemorrhage were examined.

**RESULTS:** The main causes of mortality in premature infants were severe asphyxia and its sequelae such as combined ischemic and hemorrhagic brain and spinal cord damage (45%). Severe hypoxia was confirmed by laboratory investigations. Critical lactate and acid-base blood values corresponding to decompensated lactate acidosis were registered in newborns including hyperlactatemia up to 9±3.6 mmol/l, pH 7.01±0.21, BE (-12.3) [-30; -7.9] mmol/l. The OI and OSI, indicating severity of hypoxia, were significantly elevated with their medians being 13.7[9.6;19.8] and 31.8[9.6;16.2], respectively. All infants had central nervous system lesions of varying severity with the underlying morphological immaturity. Pericellular and perivascular cerebral edema, unilateral or bilateral subcortical and subependymal hemorrhages, intraventricular hemorrhages of various degrees, up to tamponade and blood leakage into the cisterna magna, and cerebral leukomalacia were the most frequent findings. In all patients, altered neuronal shape and size as well as hyperchromic nuclei were revealed in the subcortical zone. Changes in all cell structures (cytoplasm, nuclei, and nucleoli), as well as satellitosis and neuronophagy, were characteristic. CONCLUSION: Polymorphic morphological changes were observed in the brain structures of premature infants who died as a result of perinatal hypoxia. Along with signs of morphological immaturity, irreversible changes in cortical and subcortical neurons were found. The magnitude of changes is associated with the severity of perinatal hypoxia and decompensated lactate acidosis.

**KEYWORDS** — newborn, lactic acidosis, perinatal hypoxia, brain, subependymal hemorrhage, intraventricular hemorrhage.

the formation of the structural components of the microvasculature is not yet complete. The major abnormalities of early neonatal period resulting in various CNS lesions are low vascular density of the microcirculatory system and non-uniform caliber of arterioles and venules, which significantly worsens intracerebral circulation and increases the risk of complications. At the end of the neonatal period, when the angiogenesis is still in progress, the hemodynamic imbalance persists due to the dysregulation of capillary blood flow and functioning arteriolo-venular shunts [16].

Another issue is the presence of the germinal matrix in premature infants, which can become the main source of intraventricular hemorrhage (IVH) of varying severity if exposed to negative factors [17, 18]. Such features of germinal matrix as vascular network fragility, paucity of pericytes, insufficient fibronectin in basal lamina, promote disruption of its integrity and hemorrhages. Hypoxia and ischemia, hypercapnia, acidosis, pneumothorax, respiratory distress syndrome (RDS) of the neonate, sepsis, hemostasis disorders directly affect the anatomical structures of the germinal matrix. The impact of these adverse factors enhances the cerebral blood flow fluctuations, thus contributing to IVH development [19, 20]. There is a linear correlation between the frequency of this complication and gestational age: the smaller the gestational age, the higher the risk of hemorrhage. At the same time, IVH grade 1–2, ligation of hemodynamically significant patent ductus arteriosus, and lung diseases can contribute to delayed brain development in preterm infants in the postnatal period [13].

The aim of the study was to examine the morphology of hypoxic brain injury in premature infants.

#### MATERIAL AND METHODS

This prospective study was approved by the Independent Ethics Committee of the Clinical Research Center of Immanuel Kant Baltic Federal University and was performed at the Children's Kaliningrad Regional Hospital (Protocol 14, dated October 27, 2020). The sample included 20 premature infants who died between March 2019 and December 2020. Pathologic and histologic examination protocols were analyzed. The mean gestational age of the children was 25.9±3.7 weeks, median birth weight was 650 [535; 940] grams, and height was 32.6±5.4 cm. In the sample, 17 (85%) newborns had extremely low birth weight (ELBW). Severe asphyxia was detected at birth in 19 (95%) newborns, with a median Apgar score of 2 [1.0; 3.0] points at minute 1, and 4 [2.0; 5.0] points at minute 5. At birth, the newborns received basic or advanced life support in accordance with neonatal resuscitation guidelines [21].

The following parameters were studied:

1. Clinical assessment of a newborn, which includes Apgar score at 1 and 5 minutes postpartum.

2. Clinical evaluation of the neonatal period.

3. Blood gases, the acid-base balance and lactate levels in arterialized blood using a Gem Premier 3000 analyzer (USA).

4. Brain pathology after its extraction during autopsy and dissection according to Buyalsky-Flexig technique including examination of the surface, areas of suspected malacia, infarcts, cysts, hemorrhages, malformations. The dura and pia mater, ventricles, vascular plexuses were also examined, and evidence of hydrocephalus was assessed. After examination, frontal sections of the cerebral hemispheres according to the Fischer technique were made and the samples from the following areas were taken for analysis: cortex, parietal subcortical tissue, hippocampus, striatum, cerebellum, and hemorrhage zones. After labeling the material in plastic cassettes, the routine preparation was performed, followed by placement into Histomix homogenized mixture and preparation of paraffinembedded blocks. Histological sections were stained with hematoxylin and eosin, as well as according to Nissl. Histological examination was performed using a Nikon Eclipse 55i microscope.

Statistical analysis was performed using Statistica 10 (USA) software. Arithmetic mean (M) and standard deviation (SD) were calculated for normally distributed data. Median (Me) and interquartile range (Q1; Q3) were determined for quantitative characteristics with non-normal distribution. The character of distribution was tested using Shapiro-Wilk test.

# RESULTS

### A review of the causes of fatal outcomes and laboratory criteria for severe perinatal hypoxia

The major causes of death in 9(45%) premature infants were severe asphyxia and its consequences such as combined ischemic-hemorrhagic brain and spinal cord injury, the other included neonatal ARDS and bronchopulmonary dysplasia in 6(30%), congenital infections in 3 (15%), birth trauma in 2 (10%) cases. At birth, newborns had clinical signs of severe perinatal asphyxia such as very low Apgar score and acute respiratory failure. Clinically diagnosed severe hypoxia was confirmed by laboratory tests. Critical lactate and acid-base blood values, corresponding to decompensated lactic acidosis, such as hyperlactatemia  $(9\pm3.6 \text{ mmol/l})$ , abnormal pH  $(7.01\pm0.21)$ , and BE ((-12.3) [-30.0; -7.9] mmol/l) were revealed. The neonatal period was extremely unfavorable, the measures taken did not stabilize or improve the neonates' condition. In all cases progressive deterioration resulting in death was observed. Fifteen (75%) newborns died in the early neonatal period, with a median life expectancy of 18 [11.0; 36.0] hours, and in 20 (25%) cases

the disease duration ranged from 10 to 90 days, with a median life expectancy of 19 [10.0; 28.0] days.

Brain pathomorphology in the preterm infants The pathological examination revealed that all children had brain damage of varying severity with gestational age-specific morphological immaturity. Pathological changes in the brain structures depended on the duration of antenatal hypoxia and acid-base disorders, respiratory distress syndrome of the newborn, life expectancy and had a complex character. Pericellular and perivascular cerebral edema, unilateral or bilateral subcortical (Fig. 1), subependymal hemorrhages, intraventricular hemorrhages of various degrees, including tamponade and blood leakage into the cerebellar-medullary cistern, cerebral leukomalacia were recorded in all cases.



**Fig.1.** Subcortical hemorrhage in a newborn with gestational age of 36 weeks. Hematoxylin and eosin staining.  $\times 200$ 

Histological examination revealed immature neurons in all cortical layers represented by rounded, small cells with hyperchromatic nuclei and a narrow cytoplasmic rim in preterm infants born at 22–29 weeks' gestation with ELBW (Fig. 2). The most hyperchromatic nuclei were located predominantly in the second cortical neuronal layer. As gestational age increased up to 30–33 weeks, larger differentiated neurons with distinct cytoplasm appeared in layers 3 and 5 of the cortex; pyramidal neurons also occurred.

In all cases, differentiated neurons were visualized in the subcortical area, however, along with the larger ones, clusters of smaller round-shaped neurons with hyperchromatic nuclei were found. Nucleoli were detected in some of the neurons. In this zone, neuronal nucleoli were larger and intensely stained. In some nuclei chromatin was located peripherally as small granules. The karyoplasm in these nuclei was lightly stained. Nuclei and nucleoli were located peripherally: the former were found near the outer membrane



*Fig. 2. Small rounded cortical neurons at gestational age of 29 weeks. Hematoxylin and eosin staining.* ×200

of neurons, the latter lost their central location in the nucleus and were localized near the its membrane. In some neurons, nuclei were not stained, which indicated kariolysis. Clusters of intensely stained neurons, the so-called *dark* neurons, many of which deformed with irregular contours, were detected (Fig. 3). In addition, satellitosis and neuronophagy were observed. The neuronal alterations were registered both in the cerebral cortex and subcortical structures.



*Fig. 3.* Subcortical neurons, dark neurons, pericellular and pericapillary edema at gestational age of 33 weeks. Hematoxylin and eosin staining. ×400

Nissl staining revealed non-uniform dye uptake among various neurons: the *dark* ones had more intensive stain accumulation, the others were lightly stained or had chromatolysis with Nissl dye dispersion. Many neurons demonstrated stained cytoplasmic periphery with pale perinuclear region (Fig. 4).

Cortical and subcortical capillaries were irregularly engorged, pericapillary edema and diapedetic



*Fig. 4.* Various intensity of neuronal staining at gestational age of 36 weeks. Nissl staining. ×400

hemorrhages were evident. The pia mater was swollen with its vessels engorged. The vascular plexus was engorged, swollen, and vacuolar dystrophy of epithelial cells was observed (Fig. 5).



*Fig. 5.* Vacuolar dystrophy of vascular plexus epithelial cells at gestational age og 28 weeks. Hematoxylin and eosin staining. ×1000

# DISCUSSION

Central nervous system development extends beyond the antenatal period far into the postnatal one. Any harmful exposure occurring during these periods can dramatically change the trajectory of brain development. One of the main factors negatively affecting this process is perinatal hypoxia, encompassing the antenatal, intrapartum and postnatal periods. The longer the antenatal hypoxia lasts, the more severe may be the hypoxic-ischemic injury to various parts of the brain in the fetus and the newborn [24, 25]. Severe antenatal hypoxia leads to neuronal death, which is associated with significant energy deficit and increased intracellular lactate production [26], directly affecting the increased levels of cytotoxic reactive oxygen species. This results in edema, swelling and necrosis of neurons [27, 28]. Hyperlactatemia found in children

at birth is an important marker of severe hypoxia. It is associated with such morphological signs as pericellular and perivascular cerebral edema, intraventricular hemorrhages of various severity, cerebral leukomalacia.

At the same time, decompensated metabolic acidosis is another marker confirming the severity of perinatal hypoxia [29], which also leads to neuronal necrosis [4, 30]. Acidosis impairs neuronal activity by affecting pH-sensitive channels such as acid-sensing ion channel 1a (ASIC1a) [31, 32] and N-methyl-Daspartate (NMDA) receptors [33].

In the case of preterm birth, the morphogenesis of CNS continues postnatally under special conditions, with a significantly increased risk of external factors impact. Ensuring the oxygenation of the developing brain in the postnatal period is the main therapeutic challenge in premature infants. Special attention should be focused on the study of cerebral blood flow variability, vasoreactivity and autoregulation, which differ dramatically in various periods of brain development [23]. Low cerebral blood flow and fluctuations in systemic hemodynamics combined with impaired cerebral autoregulation maintain hypoxia, which leads to varying damage to brain structures [15]. In preterm infants with gestational age less than 32 weeks, the periventricular white matter is the most sensitive to hypoxia, which can result in a specific type of damage, the so-called periventricular leukomalacia [34, 35].

As a result of long-term hypoxia and decompensated metabolic lactic acidosis, irreversible changes occur in the neurons of the cerebral cortex and subcortical structures. They affect all cell components including nucleus, nucleolus and cytoplasm.

## CONCLUSION

Morphological changes in the brain of premature infants who died as a result of perinatal hypoxia are polymorphic. Along with signs of morphological immaturity, irreversible changes in cortical and subcortical neurons can be detected. The magnitude of the alterations is associated with the severity of perinatal hypoxia and decompensated lactic acidosis.

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# SOME ASPECTS OF SURGICAL TREATMENT OF DIFFUSE PERITONITIS

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ABSTRACT — The purpose of the study was to analyze the characteristics of the course and outcome in patients with diffuse peritonitis, depending on the methods of repeated sanitation and drainage of the abdominal cavity and ultrasound monitoring. The authors carried out a retro-spective analysis of the results of treatment of 102 patients. All patients were divided into 2 groups depending on the method of treatment, with randomization by extent and phase of peritonitis. In the group 1, a semi-open method of treatment was used: sanitation and drainage of the abdominal cavity with continuous peritoneal lavage, and staged relaparotomy according to indications. In the group 2, video laparoscopic sanitation and drainage of the abdominal cavity on demand were applied. In this group, with the Mannheim Peritonitis Index below 15 points, drainage was not performed. With the Mannheim index below 20 points, 1–2 drains were installed, when above 25 points — 3–4 drains. Postoperative complications in patients of the group 1 were found in 40.9%. In the group 2, there were fewer complications compared the group 1. This was due not only to fewer local complications, but also intra-abdominal complications, which were almost 2 times fewer. Thus, video laparoscopic sanitation is the method of choice for planned repeated sanitation of the abdominal cavity. Relaparotomy should be performed only with strict indications in the event of intra-abdominal complications and if video laparoscopic sanitation cannot be performed. Ultrasound monitoring in the post-operative period makes it possible to timely identify postoperative complications, differentiate their nature and exact localization.

**KEYWORDS** — diffuse peritonitis, video laparoscopic sanitation, intra-abdominal complications, relaparotomy, relaparoscopy.

# INTRODUCTION

Despite the progress achieved in early diagnosis and modern methods of treating surgical diseases, the problem of treating peritonitis and its complications remains unresolved. The introduction in hospitals of ultrasound examination (US), computed tomography, methods of endoscopic and laparoscopic surgery, robot-assisted operations, precision surgery techniques, as well as new methods of anesthesiology, intensive therapy and detoxification did not lead to a significant decrease in complications, and hence to a progress in the treatment of diffuse peritonitis [1, 2].

Pathophysiological changes in diffuse peritonitis are characterized by excessive release of inflammatory mediators into the bloodstream, which is accompanied by widespread and long-term impairment of tissue perfusion in all organs and tissues and the formation of multiple organ dysfunction syndrome [3, 4]. According to a number of authors, one of the leading factors in DP is the formation of an enteric insufficiency syndrome with subsequent portal bacterial translocation and the formation of abdominal sepsis [5, 6, 7].

This is evidenced by the unsatisfactory results of diffuse peritonitis treatment. So, according to the literature, mortality in diffuse peritonitis ranges from 30 to 45% and increases with its purulent form up to 80% in the case of the development of abdominal septic shock and multiple organ failure syndrome. The incidence of postoperative complications, in particular, abdominal abscess is from 25 to 90%, of pneumonia — 15–32%, of external intestinal fistula — 5–13% [8, 9].

The priority in the complex treatment of diffuse peritonitis is the sanitation and drainage of the abdominal cavity. The severity of the pathological process and the need for repeated sanitation of the abdominal cavity directly depend on the quality of the primary sanitation and drainage. The surgeons'views about the choice of the least traumatic and technically simple way to eliminate the source of peritonitis are also contradictory [10, 11].

#### *Purpose of the study:*

To analyze the features of the course and outcome in patients with diffuse peritonitis, depending on the methods of repeated sanitation and drainage of the abdominal cavity and ultrasound monitoring.

## MATERIALS AND METHODS

The study included 102 patients aged from 53 to 81 years with diagnosed with diffuse peritonitis. There were 64 (62.7%) women and 38 (37.3%) men. All patients were divided into 2 groups depending on the method of treatment with randomization according to the extent and phase of peritonitis (sepsis, severe sepsis, infectious toxic (septic) shock), as well as the characteristics of the exudate in the abdominal cavity. The group 1 included 44 patients who underwent a semi-open method of treatment — sanitation and Petrov'sdrainage of the abdominal cavity with continuous peritoneal lavage, and staged relaparotomy (RL) according to indications. A solution of Chlorhexedine, Ringer and Novocaine was used for the abdominal cavitylavage. In this group, the causes of peritonitis had been: acute appendicitis in 18 (40.9%) patients, acute cholecystitis in 17 (38.6%), perforated gastric and / or duodenal ulcer in 6 (13.6%), acute colonic obstruction in 2 (4.6%), abdominal trauma with damage to the colon in 1 patient (2.3%). Serous-fibrinous exudate was detected in 31 (70.5%) patients, fibrinous-purulent and purulent — in 19 (29.5%) patients, respectively.

Group 2 included 58 patients who underwent video laparoscopic sanitation and drainage of the abdominal cavity on demand. The differentiated approach to the number of drains depended on the source of peritonitis and the calculation of the Mannheim Peritonitis Index.

In 7(12%) patients with the Mannheim Peritonitis Index below 15 points, during the primary operation after thorough sanitation of the abdominal cavity, no drainage was performed. In this subgroup of patients, the cause of peritonitis had been acute cholecystitis or perforated gastric and / or duodenal ulcer. The exudate revealed during the operation was serous-fibrinous. If the Mannheim index was below 20 points, 1–2 drainages were installed, when above 25 points — 3-4 drainages, especially with purulent exudate. Early enteral nutrition was started on days 2-3 of the postoperative period with the appearance of peristalsis, intestinal noise, and decreased discharge from the intestinal tube. The mixtures used for enteral nutrition (Nutritek, NutrienOsteo, Nutrien Immune) were administered through a naso-jejunal tube inserted along the Treitz ligament during the surgery.

In the group 2, the causes of peritonitis were acute appendicitis in 21 (36.2%), acute cholecystitis in 16 (27.6%), perforated gastric or duodenal ulcer in 9 (15.5%), abdominal trauma with damage to the large intestines in 7 (12%) and acute intestinal obstruction in 5 (8.7%) patients. The serous-fibrinous exudate was detected in 34 (58.6%), and fibrinous-purulent and purulent in 24 (41.4%) patients, respectively.

More than half of the patients sought help after 24 hours from the onset of the disease — 59 (57.8%). Within a period from 12 to 24 hours from the moment of the disease, 28 (27.5%) were hospitalized, the remaining 15 (14.7%) patients came to the hospital within 6 after the onset of the disease.

Among concomitant diseases, diseases of the cardiovascular system were in the lead, in particular, hypertension and ischemic heart disease in 68 patients (66.7%). Chronic non-specific lung diseasesranked second: chronic obstructive pulmonary disease and bronchial asthma in 18 (17.7%). Diseases of the genitourinary system (chronic pyelonephritis, prostate adenoma, urolithiasis) had been diagnosed in 12 (11.8%) patients. Diabetes mellitus, obesity, diffuse toxic goiter had been detected in 15 (14.7%) patients. Gynecological diseases had been detected in every fifth patient among all 64 women, most often being detected uterine fibroids, genital prolapse, chronic inflammatory diseases of the pelvic organs.

All patients were assessed for the functional state of organs and systems using the SO-FA scale. The assessment of the severity of the patient's condition, as well as the prognosis, was assessed by the Mannheim Peritonitis Index. The Mannheim Peritonitis Index in group 1 averaged 25.2 points, in group 2 - 24.9points.

All patients were examined according to protocols using standard laboratory biochemical methods. The fundamental methods in the diagnosis of peritonitis were: plain radiography and ultrasound of the abdominal organs. For differential diagnosis, esophagogastroduodenoscopy and laparoscopy were also used.

Preoperative preparation included correction of volemic disorders, imbalance of protein-electrolyte deficiency, therapy of concomitant diseases. All patients underwent emergency surgery according to the standard principles of peritonitis treatment.

In the postoperative period, ultrasound of the abdominal organs was performed in order to identify possible local purulent-septic complications. Monitoring was performed on days 1, 3 and 5 of the postoperative period.

The effectiveness of therapy was assessed by the normalization of clinical and biochemical parameters, protein, lipid, water-electrolyte and carbohydrate metabolism, the level of medium molecular weight peptides, leukocytic index of intoxication (LII) and improve-ment of the patient's condition.

Statistical data processing was carried out using Statistica 8.0 software packages. The statistical significance of the differences was determined using the Student's test. The critical level of significance when testing statistical hypotheses was taken equal to p < 0.05

#### **RESULTS AND DISCUSSION**

Upon admission to the hospital, patients complained of acute or dull abdominal pain, nausea and vomiting that did not bring relief, and a slight increase in body temperature. Patients hospitalized later than 24 hours from the moment of the disease onset also reported complaints of stool and gas retention. When the cause of peritonitis was a perforated gastric and / or duodenal ulcer, complaints characteristic of peptic ulcer disease were detected anam-nestically. Patients with abdominal trauma indicated when asked about a blow to the abdomen on the eve of hospitalization. The tension of the anterior abdominal wall with positive symptoms of peritoneal irritation was objectively determined in all observed patients.

The calculation of the Mannheim Peritonitis Index showed that both in groups 1 and 2 peritonitis of the 2nd degree of severity prevailed (20–30 points).

Abdominal sepsis was detected in 8 (18.2%) patients in group 1 and in 13 (22.4%)patients in group 2. Septic shock was diagnosed in 5 (11.3%) patients in group 1 and 7 (12.1%)patients in group 2. Multiple organ failure according to the SOFA scale was found in the majority of patients.

A comparative analysis of indicators of endogenous intoxication in patients of group 1 and group 2 is presented in Table 1.

As you can see from the presented table, the level

changes described above disappeared by the end of the first week.

In the case of diffuse peritonitis progression, in addition to free fluid in the abdominal cavity, anechoic areas in 2 or more anatomical areas were also determined. After 48–72 hours, anechoic areas were determined already in all parts of the abdominal cavity. Inflammatory infiltration was defined as foci of increased echogenicity without a fluid component; if abscesses were formed, anechoic or heterogeneous non-pulsating fluid formations were visualized. In such cases, sanitation of the abdominal cavity is indicated.

Postoperative complications of both groups are presented in Table 2.

Analyzing the incidence of postoperative complications in patients of the group 1, we found that 18 (40.9%) patients with intra-abdominal complications required relaparotomy. Moreover, in 11 of them, relaparotomy was performed once, in 5 patients twice, in 2 — three times. The need for sanitation was confirmed by abdominal ultrasound. A favorable picture of abdominal ultrasound (a decrease in the

Indicator	Group 1	Group 2	р
Medium molecular weight peptides on the day of hospitalization (ODU)	0,495 ± 0,023	0,509 ± 0,013	p>0,05
Medium molecular weight peptides on the day 5 (ODU)	0,398 ± 0,023	0,339 ± 0,029	p<0,05
LII on the day of hospitalization	8,79 ± 0,81	8,25 ± 0,66	p>0,05
LII on the day 5	8,81±0,63	8,79 ± 0,62	p>0,05

Table 1. Indicators of endogenous intoxication

of medium molecular weight peptides in the groups did not differ significantly on the day of hospitalization and on the day 5. LII in patients of both groups slightly increased after surgery, but by the end of the first week it did not differ from the normal values.

In patients with the third degree peritonitis by the Mannheim index (above 30 points), an increase in triglycerides was noted, however, by the end of the second week after surgery, the values of the latter approached the norm.

An ultrasound of the abdominal organs upon admission made it possible to determine not only the presence of free fluid in the abdominal cavity, but also the thickening of the intestinal wall, expansion of the small intestine (diameter and thickness). Thus, the diameter of the small intestine varied from  $28.5\pm3.6$ to  $57.3\pm4.4$  mm, the thickness — from  $3.7\pm0.4$  to  $6.3\pm0.5$  mm. The wall of the small intestine was layered and inhomogeneous. After elimination of the focus of peritonitis and sanitation of the abdominal cavity, the thickness of the intestinal wall, an increase in its echogenicity, restoration of intestinal motility) indicated that there was no need for subsequent sanitation.

10 (17.2%) patients of the group 2 with intra-abdominal complications underwent video laparoscopic sanitation by the flow-aspiration method. Sanitation and drainage of subhepatic and subphrenic abscesses was performed in 5 cases. In the remaining patients, sanitation was the final method in the progression of peritonitis. In 4 cases, the formation of an adhesive process and intestinal paresis (significant expansion) required a conversion and a relaparotomy.

In the group 2, there were fewer complications compared to the group 1. This was due not only to less local complications of 12 (27.3%) wound suppurations in the group 1 versus 10 (17.3%) in the group 2, but also intra-abdominal complications, which were almost 2 times less in the group 2. Among 7 patients of the group 2, who did not have drainage, only 2 got postoperative wound suppuration. However, due to
Table 2. Postoperative complications

Complication	Quantity in absolute values		
Complication	Group 1	Group 2	
Suppuration of the wound	12	10	
Anastomotic leakage	4	3	
Abdominal abscess (intrafilar, subphrenic, subhepatic)	6	5	
External intestinal fistula	2	-	
Eventration	2	1	
Early adhesive intestinal obstruction	3	1	
Small intestine stress ulcers	5	7	
Pulmonary thromboembolism	4	5	
Myocardial infarction	3	2	
Acute cardiopulmonary failure	8	4	
Total	49	38	

the low frequency of observations, it is not possible to draw conclusions regarding the possible refusal of drainage during the operation.

The high incidence of common complications in both groups once again confirms the literature data on unresolved issues in the treatment of peritonitis [12, 13]. Thus, the progression of cardiopulmonary failure occurred both after planned sanitation and after relaparotomy. In the early postoperative period, 21 people died: 13 (29.5%) people from the group 1 and 8 (13.8%) people from the group 2. In all cases, the causes of death were progression of multiple organ failure syndrome, myocardial infarction, and pulmonary thromboembolism.

# CONCLUSION

1) Video laparoscopic sanitation is the method of choice for planned repeated sanitation of the abdominal cavity. Relaparotomy for diffuse peritonitis should be performed only with strict indications in the event of intraabdominal complications and if it is impossible to perform video laparoscopic sanitation.

2) Ultrasound monitoring in the postoperative period makes it possible to timely identify postoperative complications, differentiate their nature and exact localization.

3) The proposed complex of therapeutic and prophylactic measures helped to reduce complications and mortality in patients with diffuse peritonitis.

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# ORIGINAL SUBMUCOSAL SUTURE IN THE TREATMENT OF GRADE IV HEMORRHOIDS DURING MUCOPEXY: CLINICAL RESULTS AND POSTOPERATIVE PAIN SYNDROME

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**ABSTRACT** — In a comparative clinical study the effect of the method of desarterization with mucopexy in the minimally invasive treatment forgrade 4 hemorrhoidaldisease on the postoperative period was studied. The authors proposed an original patented submucosal suture with a minimal tissue trauma. It enables to achieve radical desarterization while preserving the anatomical structures of the surgical anal canal. A statistically significant decrease in pain syndrome in the postoperative period was shown in comparison with the use of a conventional twisted suture, as well as a decrease in recurrence of prolapse and bleeding.

**KEYWORDS** — hemorrhoids, hemorrhoidal disease, hemorrhoidal artery ligation, rectoanal repair, mucopexy.

## INTRODUCTION

Surgical intervention HAL-RAR (hemorrhoidal artery ligation, recto-anal repair) as an effective method of minimally invasive treatment is an alternative to traditional hemorrhoidectomy for the treatment of hemorrhoidal disease (haemorrhoidaldesease - HD) for 2 [10, 14] and even 3 and 4 stages of the disease [1, 2, 3]. In most cases it combines low trauma and a comfortable postoperative period for the patient, as well as ease of implementation for the surgeon [1, 4].

The technique of the HAL-RAR procedure is currently unified and consists in the Doppler-controlled ligation on the terminal branches of hemorrhoidal vessels and mucopexy by forming a twisting suture on the mucous-submucosal layer in the area of the internal hemorrhoidal node [7, 11, 12]. At the same time, the method is not without its drawbacks in the form of postoperative pain syndrome when the anorectal line receptor apparatus is drawn into the coiled suture, as well as in a number of cases of postoperative bleeding during its eruption [8]. If at stage 3 of hemorrhoids the HAL-RAR operation has indisputable advantages over hemorrhoidectomy, the efficiency is at the level of 94–95%, then at stage 4 HD, due to the greater mass of hemorrhoidal tissue, the standard RAR demonstrates a decrease in efficiency to 89–91%, which necessitates repeated interventions already in every 10 patients [6].

These problems were identified during the accumulation of clinical material and meta-analyzes of recent years [8, 9, 13], which currently makes us reconsider the indications for the use of RAR surgery and look for options for preventing complications, especially in patients with stage 4 hemorrhoidal disease, especially in the presence of recurrent hemorrhoidal bleeding, including in comorbid patients [5].

#### The aim of research

was to improve the treatment outcomes in patients with grade IV chronic hemorrhoids by using a modified mucopexy with a submucosal suture in the lower ampullar rectum combined withtransanaldopplercontrolled desarterization of internal hemorrhoids.

# MATERIALS AND METHODS

When performing the RAR procedure, an original submucosal suture was developed and applied using only three injections with atraumatic thread with absorbable 2/0 material in the form of a triangle directed apex towards the ligated terminal branch of the hemorrhoidal artery. The main difference from the twisted suture used in the traditional RAR procedure was a decrease in the zone of ischemia of the mucous-submucosal layer when tightening the ligature, as well as improved visual control of the possible entry of fragments of the anorectal line into the suture. These two conditions were aimed at preventing complications and undesirable consequences of the RAR procedure associated with pain syndrome and possible postoperative bleeding.

The study group consisted of 68 patients operated on from January 2017 to December 2019 for grade 4 hemorrhoidaldisease. According to the classification of Yu.A.Shelygina et al. (2015), patients were classified as stage 4A hemorrhoids with a border between

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external and internal nodes. The main clinical manifestations were prolapse of internal nodes during bowel movements and physical activity and the presence of external nodes, as well as the release of blood during bowel movements and prolapse.

A prospective cohort study was carried out with the division of patients into 2 groups. In the test group, 34 patients used the "Method for minimally invasive treatment of patients with stage 3 and 4 hemorrhoids" (RF patent dated May 25, 2015 No. 2553937). The control group also included 34 patients who underwent doppler-controlled desarterization with mucopexy of internal hemorrhoids with a twisted suture. In both groups the age, gender, severity of clinical manifestations and objective criteria of hemorrhoids, the nature of concomitant diseases were comparable.

The mean age was similar in the main and control groups (main -53.5 (41.1–65.9) years, control -52.4 (37.8–67.0) years, p = 0.97), gender the structure also did not differ significantly (p = 0.16).

In the postoperative period, 24 hours and 7 days after the intervention, the severity of the pain syndrome was recorded according to the visual analogue scale (VAS) (Numerical Rating Scale, NRS). An indication for the administration of an analgesic was pain syndrome of 3 or more points on the VAS scale. The number of patients who required pain relief one day after the intervention was assessed which reflected the possibility of the patient returning to their normal activities.

Bleeding after the procedure was assessed as the presence or absence of bleeding during bowel movements on the 3<sup>rd</sup>, 15<sup>th</sup>, 60<sup>th</sup> days after desarterization with mucopexy. Possible long-term recurrence of prolapse of internal hemorrhoids was assessed 6 and 12 months after surgery. Indications for repeated interventions were also determined due to the persistence of the symptoms of falling out bleeding hemorrhoids.

Statistical processing of the obtained results was carried out by parametric and nonparametric methods, taking into account the type of distribution of quantitative data, using Student's t-test and Fisher's exact test, odds ratio (OR) with a 95% confidence interval (95% CI). Results are presented as mean (M) and standard deviation (SD). The critical value of the significance level (p) is taken as  $p \le 0.05$ .

## **RESULTS AND DISCUSSION**

The course of the early postoperative period in most patients of both groups was characterized by a moderately severe pain syndrome, in some cases there was bleeding after defecation, in some observations in the long term after the operation, a recurrence of prolapse of internal hemorrhoids was revealed. When comparing the observation groups, the intensity of pain syndrome in 24 hours after the operation, as well as on the 7<sup>th</sup> day, differed. Thus, in the main group, one day after desarterization with mucopexy, the pain index on the VAS scale was  $2.5\pm0.22$ , in the control group —  $3.47\pm0.21$  (p = 0.02). The number of patients with a VAS score of 3, which required the appointment of analgesics, was 16 in the main group and 26 in the control group one day after the intervention. The odds ratio (OR) was 3.250 (95%, CI: 0.097–0.774).

A week after the intervention, when interviewing patients of the main group, the majority of pain syndrome was absent or insignificant (on average on a scale of  $0.62\pm0.12$ ), and in the control group it remained in half of the patients at a level of 2 or more (on average  $1.35\pm0.16$ ), the differences are also statistically significant (p<0.01).

A decrease in the severity of pain syndrome after surgery is an indicator of less trauma when a submucosal suture is imposed with the possibility of visual assessment of threading without capturing the receptor zones of the anal canal. This factor increases the comfort of the postoperative period for the patient and is an important factor when choosing an intervention by the surgeon.

The discharge of blood during defecation was recorded on the 3<sup>rd</sup>, 15<sup>th</sup> and 60<sup>th</sup> days after the operation and was considered significant in the case of self-isolation of liquid blood or clots visually determined with fecal masses. It is known that after mucopexy with a grafting suture, this phenomenon is described by the authors and is considered objectively as traumatic of the intervention.

In 3 days after the intervention, bleeding was noted in 9 patients of the main group and 19 patients in the control group (OR = 0.284, 95% CI: 0.103-0.788). This indicator was considered important in the treatment tactics, since against the background of the initial anemia, which served as an indication for surgery, in 4 patients the persistence of blood loss and a decrease in hemoglobin less than 8 g/dl was an indication for blood transfusion in the hospital.

After 15 days, the number of patients with bleeding decreased and amounted to 2 in the main group and 10 in the control (OR = 0.150 (95% CI 0.030-0.749)). On the 60<sup>th</sup> day, blood secretions were retained in 6 patients of the control group, in the main group they were not noted (when assessed using Fisher's test p<0.05).

Bleeding in the postoperative period accompanies the inevitable eruption of tissues after the twisting suture, as well as when it comes off after partial resorption. The minimization of thread punctures and punctures during the submucosal suture application and its implementation outside the intestinal lumen made it possible to avoid this symptom in the main group of patients.

In 6 months after the intervention, the prolapse of internal hemorrhoids was recorded in 23.5% (8 of 34) patients after doppler-controlled desarterization with mucopexy of internal hemorrhoids according to the standard technique and in 5.9% (2 of 34) with the use of the original submucosal suture. Differences in the groups are statistically significant (p <0.05, medium strength).

In 12 months after the operative treatment, the trimmed results were studied in 55 patients (29 of the main and 26 of the control groups). It was noted that, despite the decrease in the number of examined patients, the loss of nodes was also observed in 2 patients of the main group and 8 patients of the control group. The differences were again statistically significant (p <0.05, medium strength).

It should be noted that the degree of node prolapse in the control group repeated intervention in 3 people. The remaining 5 patients in the control group and 2 patients in the main group abstained from repeated intervention, given the minor clinical manifestations of the prolapse of nodes with their independent reduction.

The effectiveness of surgical intervention in terms of recurrent prolapse indicates a more pronounced regression of the cavernous tissue in those operated on in the main group and a lower probability of repeated interventions in order to completely relieve the symptoms of internal hemorrhoids in patients with stage 4 of the disease.

# CONCLUSION

The original submucosal suture when performing the RAR procedure in minimally invasive treatment of hemorrhoids is a safe and effective element of surgical intervention in patients even with stage 4 of the disease, which makes it possible to statistically significantly reduce pain and reduce the need for analgesics in the postoperative period. After the application of the submucosal suture, delayed bleeding is not observed and the frequency of relapses in the form of prolapse of internal hemorrhoids is significantly reduced, which makes it possible to recommend it as a routine intervention and an alternative to both traditional hemorrhoidectomy and the standardized HAL-RAR intervention with a coiled mucosal-submucosal suture.

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# A NOVEL METHOD FOR SURGICAL TREATMENT OF OSTEOMYELITIS OF DISTAL PHALANX: A CLINICAL CASE

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ABSTRACT — BACKGROUND: Osteomyelitis is an infectious-inflammatory destructive bone disease caused by nonspecific and specific microflora, or microbial communities. Diabetes mellitus is the most common pathology associated with osteomyelitis and may be responsible for exacerbation of chronic osteomyelitis. OBJECTIVE: to demonstrate a new patented technique for surgical treatment of osteomyelitis of distal phalanx (I finger) on the example of a clinical case.

METHODS: A female patient P. 34 years old, who has type 2 diabetes mellitus, decompensated. Daily glucosometry shows changes in capillary blood glucose from 7 to 25 mmol/L during the last month; the last blood test for HbA1c — 10.4%, it should be noted that the patient adheres to the diet prescribed by the endocrinologist and receives drug therapy according to treatment standards, however, attempts to compensate the disease have been failed. The patient suffers from obesity of 1 degree (BMI = 30.11). **RESULTS:** Operation: after preparation of the surgical site with Betadin's solution, local anesthesia according to Oberst-Lukashevich (injection of 3 ml 2% Lidocaine solution on each side into the lateral surfaces of the I finger of the right hand from the place of harness application, proximal to the intervention area) was provided. Extraction of the nail plate with dissection of soft tissues along the phalanx and along the projection of the nail bed and rotation of tissues downwards to expose the bone destruction focus. Then necrectomy of the damaged bone was performed followed by sanitization and returning of soft tissues to their place without suturing. CONCLUSION: The given example of surgical treatment of finger osteomyelitis has undeniable advantages due to a number of reasons: elimination of traumatization of finger's working surface, achievement of cosmetic effect, preservation of aesthetic and function of hand, as well as all types of sensitivity of finger skin. It is worth noting that this method of surgical treatment of osteomyelitis has more

favorable course of the postoperative period due to a shorter duration of pain syndrome and the general reaction of the body.

**KEYWORDS** — Osteomyelitis, distal phalanx, surgery, aesthetics.

# RELEVANCE

Osteomyelitis is an infectious-inflammatory destructive bone disease caused by nonspecific (S. aureus, S. epidermidis, less often E. faecalis, P. aeruginosa, E. coli, etc.) and specific microflora (M. tuberculosis, T. pallidum, etc.), or microbial communities. The most common osteomyelitis causative agent is golden staphylococcus, which was isolated from pus of patient with osteomyelitis by French microbiologist and chemist Louis Pasteur in 1880. Pathogenic microorganisms can get into the bone tissue in the result of injury of soft tissues located in close proximity to the bones; through damaged joint tissues and open fractures, as well as from contiguous or distant focus of infection (for example, in case of tonsillitis).

Finger osteomyelitis, or bone panaritium, is a result of dissemination of the pathological process from the soft tissues of the finger to the bone. Signs of osteolysis can be determined only by the end of the 2<sup>nd</sup> or beginning of the 3<sup>rd</sup> week of the disease on finger radiograph images. Visualisation and treatment of osteomyelitis remains difficult, because of depending on the age of patient, type of inflammatory process (acute or chronic), way of spreading (hematogenic or non-hematogenic), as well as on the state of the vessels of any anatomical region and on the immune status of the patient [1]. It is known that reduced immune resistance contributes to the development of infectious-inflammatory disease and the addition of secondary infection.

Various disorders of the humoral immune system (low level of complement factor C4, decreased cytokine activity after stimulation) are described in patients with type 2 diabetes mellitus [2].

Dysfunction of immune response is caused by hyperglycemia which characterizes diabetes mellitus [3]. Diabetes mellitus is a chronic endocrine-metabolic disease that develops due to impaired secretion and/or action of insulin in tissues, attributed to hyperglycemia and disruption of all types of metabolism leading to various complications. Blood examination for glycat-edhemoglobin (glycohemoglobin, HbA1c) is the most important diagnostic sign and the dominant indicator of chronic glycemia [4]. Blood glucose measurement, unlike HbA1c, shows its level only at the time of the study, while HbA1c provides information on the aver-

age glucose level over the past 8–12 weeks [5]. In patients with diabetes mellitus, level of HbA1c depends on the level of hyperglycemia: the higher the level of HbA1c, the higherglycemia in recent months is and, accordingly, the risk of developing complications of diabetes mellitus is greater.

Therefore, in patients with diabetes mellitus, HbA1c is used as a predictor of risk of microvascular complications [5].

Metabolic disorders in diabetes mellitus affect various organ systems [6]. The most frequent and multiple are diseases of the musculoskeletal system as the result of microangiopathy, pathological changes in connective tissue and peripheral nerves associated with chronic hyperglycemia. Their prevalence in patients is usually correlated with poor glycemic control. Although the most of musculoskeletal complications of diabetes mellitus are usually not life-threatening, they often cause serious diseases and worsen the quality of life of patients [7, 8]. Diabetes mellitus is the most common pathology associated with osteomyelitis and may be responsible for exacerbation of chronic osteomyelitis [9].

Aim:

to demonstrate a new patented technique for the surgical treatment of osteomyelitis of distal phalanx (I finger) on the example of a clinical case.

## MATERIALS AND METHODS

A female patient P. 34 years old has decompensated type 2 diabetes mellitus. Daily glucosometry shows changes in capillary blood glucose from 7 to 25 mmol/L during the last month; the last blood test for HbA1c — 10.4%. It should be noted that the patient adheres to the diet prescribed by the endocrinologist and receives drug therapy according to treatment standards, however, attempts to compensate the disease have been failed. The patient suffers from obesity of 1 degree (BMI = 30.11).

The study took place at Polyclinic No. 1 of the Municipal clinical hospital No. 7 in Tver (Russia), where the patient first saw a surgeon with complaints of pain and swelling in the area of the distal phalanx of the first finger of the right hand.

The surgical method used is covered by patent RU 2745028 C1 18.05.2020, https://www.elibrary.ru/ item.asp?id=45807088.

# **RESULTS AND DISCUSSIONS**

During the first contact with the surgeon, the patient was complaining for pain of the area of the first right finger, which appeared about two weeks ago after an injury at home. The patient did not seek for medical help before. At the moment of examination, the patient's body temperature was 37.5° C. Status localis: the distal phalanx of the first finger of the right hand is swollen, skin in the phalanx is hyperemic, during palpation and movement there is acute pain. Fluctuance is observed in the area of the medial nail fold, which indicates the presence of a purulent focus. Panaritium of the I finger of the right hand was diagnosed. Based on the anamnesis and examination of the patient, surgery was proposed. After obtaining the patient's informed consent it was carried out.

Surgery plan: preparation of the surgical site with Betadin's solution, local anesthesia according to Oberst-Lukashevich (injection of 3 ml 2% Lidocaine solution on each side into the lateral surfaces of the I finger of the right hand from the place of harness application, proximal to the intervention area) was provided. Purulent focus was opened with the release of thick yellow purulent content; repeated treatment of the surgical site and application of an aseptic bandage with Levomecol ointment (chloramphenicol + methyluracil). Daily bandages with Levomecol ointment (chloramphenicol + methyluracil) were prescribed. Next day, the control radiograph image of the I finger of the right hand was performed, the image determined decrease of bone density, structure heterogeneity of the nail phalanx of the I finger of the right hand, the articular surface is not changed, preserved and clear. Soft tissues are infiltrated.

**Report**: osteomyelitis of the distal phalanx I finger of the right hand. It was decided to carry out conservative therapy, oral antibacterial drugs were added to the bandages – Lincomycin 250 mg 2 tablets 3 times a day for 1 month. Bandages with antibacterial drugs were carried out daily, minor serous secretion from the wound was noted.

After 2 weeks against the background of conservative therapy, the patient felt herself worse, I finger of the right hand swelled, hyperemia and pain during movement appeared. The symptoms were eliminated after a week of daily bandages. I finger of the right hand was radiographed, total destruction (sequestration) of the terminal phalanx I finger of the right hand except articular surface was determined. Conclusion: osteomyelitis of the distall phalanx I finger of the right hand.

Based on the anamnesis and examination of the patient, surgery was proposed. After obtaining the patient's informed consent it was carried out. After preparation of the surgical site with Betadin's solution, local anesthesia according to Oberst-Lukashevich(injection of 3 ml 2% Lidocaine solution on each side into the lateral surfaces of the I finger of the right hand from the place of harness application, proximal to the intervention area) was provided. Extraction of the nail plate with dissection of soft tissues along the phalanx and along the projection of the nail bed and rotation of tissues downwards to expose the bone destruction focus. Then necrectomy of the damaged bone was provided followed by sanitization and returning of soft tissues to their place without suturing.

It was prescribed to continue antibacterial therapy (Linkomycin 250 mg 2 tablets 3 times a day for 10 days), bandages with Levomecol ointment (chloramphenicol + methyluracil) and Betadin solution (povidone iodine). On the control radiograph image of the first finger of the right hand a defect of the nail phalanx, the remaining base of the phalanx was determined. Contours were unclear, structure is heterogeneous, soft tissues are infiltrated. Conclusion: phalangeal osteomyelitis. Condition after surgical treatment (Fig. 1).

For the first time during the surgery shape and size of the finger were maximally preserved (Fig. 2). In addition, the novelty is closure of the postoperative scar with a growing nail plate, and therefore the traumatization of the working surface of the finger will be eliminated in future. Application of the proposed technique made it possible to reduce duration of pain syndrome in postoperative period, to carry out prevention of risk of disturbance of sensitivity of surgical site, development of scar deformation and limitation of mobility. The obvious advantages of this operation are the cosmetic effect which allows to preserve the aesthetic function of the hand, which is especially important for the female sex. In addition, the support function of the finger is preserved due to the nail plate, as well as the preservation of all types of sensitivity of the skin of the finger.



Fig. 1.



# CONCLUSION

The given example of surgical treatment of finger osteomyelitis has undeniable advantages due to a number of reasons: elimination of traumatization of finger's working surface, achievement of cosmetic effect, preservation of aesthetics and hand function, as well as all types of sensitivity of finger skin. It is worth noting that this method of surgical treatment of osteomyelitis has a more favourable course in the postoperative period due to a shorter duration of pain syndrome and the general body reaction.

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# IS IT POSSIBLE TO PREDICT POSTOPERATIVE ABDOMINAL COMPLICATIONS BY LASER DOPPLER FLOWMETRY?

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**ABSTRACT** — In this study, the authors chose to assess the prognostic capabilities of the laser Doppler flowmetry method for postoperative complications in patients with advanced peritonitis. A retrospective randomized controlled trial (evidence level IIB) was performed. The study involved 54 patients who were operated on for secondary widespread fibrinous-purulent peritonitis. The patients were divided into two groups with 27 patients in each: the main group - with developed postoperative complications and a comparison group — without complications. The research was carried out by the analyzer "Lazma MC-1" (Russia). Scanning of the parietal and visceral peritoneum, small and large intestines, as well as the edges of the laparotomic wound was performed. A total of 504 scans were performed. The relationship of indicators was determined using the Spearman's rank correlation coefficient. Microcirculation indices in both groups were characterized by pronounced disorders of pre- and postcapillary resistance and disorders of tissue perfusion. The following indicators turned out to be statistically significant: percentage of microcirculation  $(r = 0.77 \text{ at } p \le 0.05)$ , standard deviation  $(r = 0.67 \text{ at } p \le 0.05)$ , coefficient of variation (r = 0.59 at  $p \le 0, 05$ ) and shunting percentage. In patients of the main group, the changes were expressed in an increase in pre- and postcapillary resistance, progressive arterio-venular shunting. All this ultimately led to disturbances in the perfusion of the intestinal wall, peritoneum and laparotomic wound, which led to postoperative complications. The technique of abdominal laser Doppler flowmetry can be a prognostic method that allows predicting the development of early postoperative complications after primary surgery and making appropriate adjustments to the treatment.

**KEYWORDS** — surgical site infections, predicting postoperative complications, laser Doppler flowmetry, abdominal microcirculation, a randomized controlled trial.

# INTRODUCTION

Surgical site infections (SSI) are a national priority in health research worldwide. The process of wound infection is complex and involves the interaction of several biological pathways at the molecular level. The incidence of postoperative infectious complications, even in developed countries, is 3-5%. Wound infections are the cause of high morbidity and mortality and can be as high as 33%. Current data indicate that surgical wound infections account for over two million nosocomial infections in patients hospitalized in the United States [1, 2, 3]. Many factors have been described that affect the prognosis of patients with SSI, including advanced age, poor nutrition, obesity, pre-existing medical conditions, immunosuppression, diabetes mellitus, advanced peritonitis, occurrence of septic shock, poor source control, organ failure, prolonged hospitalization prior to therapy, and infection with nosocomial pathogens, etc. [4, 5, 6].

Early prognostic assessment of complicated intra-abdominal infections is crucial for assessing the severity and making decisions about the aggressiveness of treatment [7]. In the postoperative period, the main therapeutic approach is to normalize the parameters of systemic hemodynamics using mainly vasoactive drugs and fluids. It is believed that the improvement in microcirculation will lead to a parallel improvement in microcirculation. However, several studies on microcirculation in critical condition have found that persistence of microcirculatory changes can occur independently of systemic hemodynamic parameters, and that such loss of coherence is associated with poor outcome [8].

Over the decades, new instruments and methods for assessing microcirculation in critically ill patients were developed. In recent years, works on the use of laser Doppler flowmetry for intestinal obstruction, ischemic bowel damage and peritonitis have appeared in the scientific literature [9, 10, 11].

Lack of works devoted to predicting postoperative complications in generalized peritonitis using laser Doppler flowmetry (LDF) prompted this study.

### MATERIAL AND METHODS

A retrospective randomized controlled trial (evidence level IIB) was performed. The object of the study was 54 patients who were operated on for secondary widespread fibrinous-purulent peritonitis in the period 2015–2020. Primary operations were carried out for

destructive diseases or injuries of the abdominal organs resulting from injuries. The duration of the disease ranged from 6 to 78 hours. The average age of the patients was  $52 \pm 4.2$  years; there were 22 men, and 24 women. Criteria for inclusion in the study: severity of the condition according to APACHE II  $\geq$  10 points; SOFA  $\geq 8$  points. Exclusion criteria: peritonitis caused by impaired mesenteric circulation; peritonitis, accompanied by persistent arterial hypotension associated with extra-abdominal causes (myocardial infarction, stroke, etc.); decompensated septic shock; peritonitis associated withstage IV malignant neoplasms and abdominal carcinomatosis. All patients were divided into two equal groups (23 patients each), the main one — with developed postoperative complications and the comparison group — without complications. The patients were comparable by sex, age, severity of pathology, as well as the severity of diabetes mellitus. Postoperative complications in the main group were distributed as follows: local suppuration of the laparotomic wound — 12; incompetence of sutures of intra-abdominal anastomoses — 5; intra-abdominal abscesses -5; eventration -4; perforation of acute ileal ulcer — 1. According to the Clavien-Dindo classification, the distribution of postoperative complications was as follows: I-5; II-3; IIIa-5; IIIb-8; IVa-3; IVb-1; V-2.

All patients after laparotomy underwent LDF with a microcirculation and microlymph flow analyzer using the Lazma MC-1 apparatus (Russia). For the purpose of objectification, the indicators were taken within 1 min from 6 points: parietal peritoneum in the area of the focus; parietal peritoneum as distant as possible from the focus; jejunum (40 cm from Treitz's ligament); large intestine (middle third of the transverse colon); on both edges of the laparotomic wound (Fig. 1).We studied: the percentage of microcirculation (PM; c.u) — which is a function of the concentration of erythrocytes in the probed tissue volume and their average velocity; standard deviation (SD; o, c.u.), i.e. average fluctuations in perfusion relative to the average value of PM — characterizing the temporal variability of perfusion and reflects the average variability of blood flow in all frequency ranges. Another indicator was the coefficient of variation (Cv;%) — it characterizes the relationship between the variability of perfusion with the average perfusion in the probed area, that is, it reflects the vasomotor activity of the vessels. In addition, the indicators obtained as a result of the wavelet transformation were also analyzed - neurogenic tone (NT), myogenic tone (MT), shunting percentage (SP) and microcirculation efficiency index (MEI). Data are presented as arithmetic mean and arithmetic mean error (M + m). The results obtained



Fig. 1. Laser Doppler flowmetry (scan of the jejunum)

were compared with the reference values obtained in patients with elective surgery for non-inflammatory diseases of the abdominal organs (n = 30). Thus, 504 scans were performed in all groups.

Statistical relationships between indicators were assessed using Basic Statistics and Tables STATIS-TICA 6.0, regression analysis, analysis of variance, and multivariate statistics. The method of adaptive randomization was used. In order to determine the significance of p differences between groups, the Student's t test was used. Differences were considered statistically significant at  $p \le 0.05$ . The relationship of indicators was determined using the Spearman's rank correlation coefficient.

# **RESULTS AND DISCUSSION**

Microcirculation indices obtained with LDF in both groups were characterized by pronounced disorders of pre- and postcapillary resistance and disorders of tissue perfusion. In the main group, the values of PM, SD and Cv remained significantly lower in comparison with the comparison group ( $p \le 0.05$ ). MT and NT of peritoneal vessels, as well as MEI in the main group was comparatively lower, however, no statistically significant difference was found (p > 0.05). At the same time, SP in the main group increased statistically significantly ( $p \ge 0.05$ ) (Table 1).

In order to identify and assess the tightness of the relationship between the two series of compared basic quantitative indicators of microcirculation (PM, SD and Cv) in the study groups, the Spearman rank correlation coefficient was calculated, which allows checking the heteroscedasticity of random errors in

Indicators	Reference values (n=30)	Main group (n=27)	Comparison group (n=27)
PM (p.u)	32,21±1,18	17,6±1,55*	23,37±1,83*
SD (p.u)	5,42±0,33	2,03±0,19*	3,69±0,15*
Cv (%)	12,64±1,50	6,03±3,79*	11,34±4,04*
NT (p.u)	0,59±0,08	0,77±0,02	0,44±0,05
MT (p.u)	0,57±0,04	0,75±0,05	0,55±0,03
SP (p.u)	1,15±0,09	1,86±0,05*	1,18±0,03*
MEI	1,22±0,03	1,11±0,09	1,29±0,11

Table 1. Indicators of abdominal microcirculation in the study groups

**Note:** \* — indicators with the value of changes p < 0.05; p.u. — perfusion units; %

the regression model. In the study of PM, there was a direct correlation with a high closeness of communication in the study groups (r = 0.77 at  $p \le 0.05$ ). (Fig. 2).

When carrying out the relationship in terms of Cv, data were obtained indicating a moderate direct correlation in the study groups (r = 0.67 at  $p \le 0.05$ ). (Fig. 3).

The study of the correlation relationship in terms of SD in the study groups revealed a direct relationship with a moderate closeness of communication (r = 0.59 at  $p \le 0.05$ ). (Fig. 4).

Currently, there are no objective methods that would reliably predict postoperative complications. The prognostic risk scales of surgical site infections developed on the basis of meta-analyzes (ASA, CDS, SSIRS, qSOFA, etc.) are often quite massive and of little use in urgent surgery. This undoubtedly requires a search for alternative methods for predicting postoperative complications in patients with urgent intraabdominal pathology [12].

A study on the abdominal microcirculation in generalized peritonitis showed significant disorders, which were expressed in changes in pre- and postcapillary resistance, leading ultimately to disorders of perfusion of the intestinal wall, peritoneum and laparotomic wound. A statistically significant decrease in PM and SD values indicated a decrease in the concentration of erythrocytes, suppression of perfusion fluctuations, and the Kv indicator showed a decrease in vasomotor activity of the vessels. Progressive arteriovenular shunting further aggravated cellular hypoxia, which was confirmed by changes in the SP index. All of the above changes led to postoperative complications. The study has shown that LDF can be a prognostic method that allows predicting the development of postoperative complications after primary surgery and making corrections in treatment.



Fig. 2. Spearman's rank correlation coefficient for the PM parameter in the study groups (r = 0.77 at p < 0.05)



Fig. 3. Spearman's rank correlation coefficient for the Cv parameter in the study groups (r = 0.67 at p < 0.05)



Fig. 4. Spearman's rank correlation coefficient for the SD parameter in the study groups (r = 0.59 at p < 0.05)

# CONCLUSION

The data obtained as a result of a retrospective randomized controlled trial allow us to conclude that

the abdominal LDF technique allows predicting early postoperative complications in patients with advanced peritonitis. The data can be used to develop an objective predictive system.

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# MALE INFERTILITY ASSOCIATED WITH OBESITY

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**ABSTRACT** — Obesity should be considered as a separate risk factor that contributes to the development of male infertility.

AIM. To assess obesity impact level on semen disturbances. Methods. The study included 130 males with fertility disorders, who, depending on the level of body mass index (BMI), were divided into two groups. The first group included 67 men with a BMI of  $22,7\pm2,9$  kg/m<sup>2</sup>. The second group included 63 men with  $38,6\pm4,3$  kg/m<sup>2</sup> (p <0.05). RESULTS. The significant tendency to decrease in the levels of LH, FSH, testosterone and an increase in the level of estradiol in men with an increase in BMI compared to men with normal weight was established (p<0.05). In addition, obese men sperm motility was 8.2% worse (p<0.05). CONCLUSION. In men with obesity and fertility disorders, the concentration and number of motile forms of spermatozoa are significantly lower compared to men with a normal body mass index.

**KEYWORDS** — male infertility, obesity, Body Mass Index (BMI), ejaculate, spermatozoa concentration, asthenozoospermia.

## INTRODUCTION

Every tenth married couple faces the problem of infertility during their reproductive life [1]. Moreover, male infertility accounts for 30–50% [2, 3]. In recent years, due to the high interest in andrology, many factors of the pathogenesis of male infertility have been clarified: varicocele, infections, oxidative stress, genetic mutations, repair of inguinal hernias, etc. [4, 5, 6,7]. One of the factors that affects sperm quality is obesity [8].

It has been established that about two billion people in the world are overweight, a significant part of which are men of reproductive age. Among the different types of obesity, abdominal obesity has the most pronounced negative effects. It is abdominal obesity that is closely associated with the risk of developing cardiovascular diseases, type 2 diabetes mellitus, metabolic disorders, erectile dysfunction and Received 19 November 2021; Received in revised form 6 December 2021; Accepted 8 December 2021

Aim

male reproductive health disorders. [9] However, until now there is no unified view on the effect of obesity on spermogram indicators.

To assess obesity impact level on semen disturbances.

#### **METHODS**

We examined 130 men over the age of 18 with male infertility. Exclusion criteria from the study: cryptorchidism, varicocele, hydrocele, sexually transmitted diseases, genetic abnormalities, thyroid diseases, primary hypogonadism. When examining patients, we took into account their height, weight, body mass index (BMI). In all men, we examined the ejaculate according to the WHO 2010 standard. Blood hormones were also tested: luteinizing hormone (LH), folliclestimulating hormone (FSH), thyroid-stimulating hormone (TSH), total testosterone, progesterone, estradiol, cortisol.

The men were distributed into 2 groups depending on the BMI indicator:

 $1^{st}$  group (n=67) — men with impaired fertility and normal body mass index (20–24,9kg/m<sup>2</sup>).

 $2^{nd}$  group (n=63) — with impaired fertility and obesity (body mass index >30 kg/m<sup>2</sup>).

The statistical analysis was performed using spreadsheets "EXCEL" and "STATISTICA 8.0". Statistical processing was carried out with the calculation of arithmetic mean values (M) and their errors (m). Differences were considered significant at p <0.05.

## RESULTS

The average age of all patients (n=130) ranged from 18 to 50: in the first group of patients it was  $34.6\pm4.8$  years, in the second group —  $32.1\pm6.2$  years (p>0.05). BMI indicators in men of both groups are shown in Figure 1. As it's shown in the Fig. 1, the BMI in patients of the second group was 1.7 times higher than in men of the first group (p<0.05).

When analyzing hormonal changes in obese patients, we found a clear relationship between the level of sex hormones and BMI. With an increase in BMI, the more pronounced decrease in the level of LH, FSH, testosterone and an increase in the level of estradiol was noted (p<0.05) (Table 1).

Table 2 shows the indicators of the ejaculate in infertile men with obesity and normal BMI. The data



*Fig.1.* BMI indicators in patients of the first and second groups (p<0.05)

Hormone indicators	1group, (n=67)	2 group, (n=63)	р
LH (IU/ml)	4,6 ± 0,4	2,9 ± 0,5	p<0,05
FSH (IU/ml)	5,7 ± 0,8	4,4 ± 0,4	p<0,05
TTH, IU/L	2,0 ± 0,1	2,2 ± 0,2	p >0.05
Total testosterone (nmol/L)	19,2 ± 2,1	11,2 ± 0,8	p<0,05
Progesterone, nmol/L	1,3 ± 0,2	1,4 ± 0,3	p >0.05
Estradiol, nmol/L	88,3 ± 5,7	114,7 ± 3,9	p<0,05
Cortisol, nmol/L	351,2 ± 25,2	348,5 ± 24,8	p >0.05

 Table 1. Serum hormone levels in infertile men with obesity and normal BMI

negative effect on hormonal balance. Due to disorders in the hypothalamus-pituitary-gonad axis, testosterone levels decrease and estradiol levels increase. As well as a violation of the ratio of androgens and estrogens due to peripheral aromatization of testosterone in adipose tissue[2]. Secondly, an increase in the amount of fat in men can lead to an increase in the temperature of the scrotum, which contributes to a violation of spermatogenesis in the testicle and the maturation of germ cells in the appendage of the testicle [8]. And the last, an increase in the volume of adipose tissue can cause a violation of the amount of proteins that should ensure the normal viscosity of the ejaculate and the mobility of sperm in it.

Clinical studies show that in obese men, the risk of male infertility occurs two times more often than in men with normal weight [2].

A meta-analysis of 25 studies showed that in men with an increase in BMI for every five units, the parameters of the ejaculate will decrease: the total number of spermatozoa, sperm concentration and sperm volume by 2.4%, 1.3% and 2.0%, respectively [8].

We have found that there is a direct relationship between the patient's weight and the indicators of LH, testosterone, estradiol. This means that the higher a man's BMI, the more the concentration of sex hormones changes. Our study showed that in men with a BMI above normal, the proportion of actively motile spermatozoa decreases (p < 0.05).

Semen analysis indicators		1group, (n=67)	2 group, (n=63)	р
Spermatozoa concentration		26,9±4,6	18,2±4,9	p<0,05
Ejaculate volume (ml)		3,7 ± 1,8	2,8 ± 1,2	p>0,05
рН		7,2 ± 0,2	7,4 ± 0,3	p>0,05
Spermatozoa motility	A+B (%)	34,8±1,5	26,6±1,3	p<0,05
	C (%)	26,8 ± 3,4	31,5 ± 3,6	p>0,05
	D (%)	38,4 ± 4,9	41,9 ± 5,2	p>0,05
The proportion of normal sperm forms (%)		18±1,2	16,5±1,7	p>0,05
Leukocyte number (mln/ml)		0,6 ± 0,1	0,5 ± 0,3	p>0,05

Table 2. Indicators of the ejaculate in infertile men with obesity and normal BMI

obtained by us indicate a clear role of obesity in the pathogenesis of ejaculate fertility disorders. In the presence of obesity, more pronounced changes in spermogram parameters were observed: a decrease in the concentration of germ cells and a violation of their motility (p<0.05).

# DISCUSSION

The pathogenesis of male infertility linked to obesity has many factors. Firstly, obesity can have a

# CONCLUSION

Obese males with fertility disorders most often have a low concentration and number of motile forms of spermatozoa compared to men with a normal body mass index. Therefore, for the treatment of pathological disorders in sperm in obese men, it's important to measure body weight and use drug therapy with aromatase inhibitors and antiestrogens.

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# OPPORTUNITIES OF PLATELET RICH PLASMA APPLICATION IN THE TREATMENT OF SKIN SCARS

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**ABSTRACT** — The number of patients with hypertrophic skin scars is increasing every year. Scar tissue is formed as a result of healing of a wound defect. Therefore, in order to develop optimal methods for the treatment of scarring skin changes, it is necessary to understand the pathogenesis of the wound process. The purpose of this work was to analyze the literature data on the effectiveness of the use of plateletrich plasma (PRP) in the treatment of scarring that appeared in patients with acne. PRP contains epidermis growth factor, vascular endothelial growth factor, which help accelerate the healing of skin wounds.

CONCLUSION: PRP can be successfully used in the treatment of atrophic scars on the skin after acne. Therefore, this method can be used both in monotherapy and in combination with surgical methods of treatment.

**KEYWORDS** — acne, scars of skin, platelet rich plasma, treatment.

# INTRODUCTION

Today, from the total number of people who need treatment for cicatricial skin changes, about 18% are patients with keloid scars, about 8% are patients with hypertrophic scars, and about 14% are patients with hypotrophic scars [1]. The number of such patients increases every year. The most common type of cicatricial skin changes are post-acne scars, or atrophic scars.

Acne inflammation is the primary process that precedes follicular hyperkeratosis. Signs of inflammation persist from the appearance of acne (in the form of inflammatory lesions) to secondary post-inflammatory changes in the skin: post-inflammatory erythema (PIE), post-inflammatory hyperpigmentation (postinflammatory hyperpigmentation, PIH) and scars. Both PIE and PIH are signs of inflammation. Due to the increased blood supply in the area of inflammation and the high permeability of the capillary walls, immune cells rush into these tissues. Scars are the result of impaired healing after inflammation, characterized by the presence of inflammatory cellular infiltrates, and are found in 77% of atrophic scars [2].

To date, dermatologists use a combined approach in the treatment of scars on the skin after acne. At the first stage it is necessary to carry out a comprehensive assessment of post-acne scars by estimation ofcicatricial erythema, type, depth and location of scars. The treatment of acne scars should be carried out according to an algorithm that includes the impact on each stage of the scarring process. After elimination of cicatricial erythema, it is necessary to influence the atrophic scar itself.The approach is determined by the type of scars and their prevalence: generalized spread or local foci [1].

It is considered that it is necessary to treat all active manifestations of acne before proceeding with the treatment of atrophic scars so as not to cause an activation of lesions in those areas where the treatment has already done.

Given the relationship between the severity and duration of inflammation in the development of scars, early treatment of acne is the best approach to prevent the appearance of acne scars. Therapy should be carried out until the inflammatory component is entirely eliminated and stopped in the absence of new rashes [3].

In recent years, a fundamentally new approach is offered for the treatment of skin scars using plateletrich plasma (PRP), an autologous blood component in which the concentration of platelets is in 3–5 times higher than the normal concentration of platelets is in the blood [4]. To date, more than 40 commercial systems have been proposed for obtaining PRP from whole blood, in most of them PRP is obtained by double centrifugation. Moreover, the final platelet concentration depends not only on the system used, but also on several individual characteristics of the patient, such as age and associated diseases [5].

The PRP properties are based on the use of the activity of some growth factors that are secreted by platelets after their activation. These factors, which are predominantly found in alpha granules, regulate key cellular processes, including chemotaxis, mitogenesis, and cell differentiation. Growth factors directly stimulate the migration of mesenchymal and epithelial cells, their division, the synthesis of collagen and other components of the intercellular matrix. Many growth factors interact with each other and activate various intracellular signaling pathways, which are necessary for regenerative processes [6].

Studies of the use of PRP for skin rejuvenation have shown that its intradermal injections improve a state of post-acne scars. It is established that the local administration of PRP has a good effect when used together with microneedle therapy for post-acne scars. This is causedby the microneedle effect on the skin that promotes better penetration of PRP and enhances the effect of platelets to boost of wound healing. It was established that the use of plasma both in the form of an intradermal injection and in the form of applications after using an ablative  $CO_2$  laser improved the recovery of laser-damaged skin and improved the clinical appearance of post-acne scars compared to the control group [7].

In the study evaluated the efficacy of the combined use of PRP and fractionated laser (FL). Patients participating in the study with scars after injuries were divided into 3 groups. Patients of the 1st group underwent transplantation of adipose tissue with the PRPadministration.FL was used in the 2<sup>nd</sup> group. Patients of the 3<sup>rd</sup> group received combined treatment using both methods [8].

In another study, the purpose of which was to study the PRP efficacy in the treatment of scars, patients of the 1<sup>st</sup> group underwent transplantation of adipose tissue without PRP, patients of the 2<sup>nd</sup> group underwent transplantation of adipose tissue 7–10 days after the preliminary administration of PRP, and patients of the 3<sup>rd</sup> group had only transplantation of adipose tissue on one side and transplantation of adipose tissue in combination with the use of PRP on the contralateral side [9]. In all studies, higher efficacy of the combined use of PRP and adipocytes was demonstrated.

The efficacy of the PRP use has been studied in the treatment of post-acne scars. Patients were prescribed laser therapy with erbium FL (EFL), then — PRP locally (in gel form). A 50% improvement was observed in 68% and 91% of patients after the 1<sup>st</sup> and 3<sup>rd</sup> courses of treatment, respectively [10]. In this study, treatment of different parts of the face was not carried out using different methods in the same patient.So, it is not possible to determine which method was more effective. In another study, patients with post-acne scars after a course of laser therapy with an ablative fractional CO<sub>2</sub> laser were given PRP injections on one half of the face and physiological saline injections on the opposite side [11]. The PRP use was accompanied by a decrease in erythema duration from  $10.4\pm2.7$  to  $8.6\pm2.0$  days. In addition, against the background of the PRP use, erythema was significantly less pronounced already on the 4<sup>th</sup> day (according to chromometry), and the duration of the presence of edema decreased by about 1 day. The PRP use has contributed, according to independent expert dermatologists, to a significantly more pronounced improvement in the overall scoring of treatment efficacy compared to the corresponding level of assessment on the control side. The average improvement was  $2.7\pm0.7$  points after applying the PRP and  $2.3\pm0.5$  points on the control side [11].

A comparison was also made of the efficacy and safety of PRP in the form of injections and for topical local application after therapy with FL for post acne scars. Patients were randomized into 2 groups. In the 1st group after FL, PRP was administered intradermally in one half of the face and physiological saline in the other one. In patients of the 2<sup>nd</sup> group after FL, on one half of the face, PRP was administered intradermally, on the other, it was applied superficially. 3 courses of treatment were carried out with an interval of 1 month between courses. Efficacy assessment was carried out 6 months after the start of treatment. It was found that the PRP use, both in the form of injections and topical, was accompanied by a shorter recovery period and significant improvements in clinical indicators [6].

Study of scar depth using optical coherence tomography showed that when using only FL for their treatment, a less pronounced improvement was noted in comparison with the combined therapy of FL and PRP (both with external and intradermal plasma application). There were no significant differences in clinical efficacy with intradermal administration and external PRP use.But it was noted that external PRP use was better tolerated, that is, it was safer [12].

The possibility of PRP using in the treatment of striae — dermal scars with epidermal atrophy arising from prolonged skin stretching — has also been demonstrated. In one of the works, such patients underwent combined treatment — radiofrequency thermal lifting in combination with PRP — once every 4 weeks [7]. The efficacy of the separate PRP use and radiofrequency thermal lifting has not been studied. After 12 weeks of treatment, the results were evaluated by a specialist using a quartile scale. All of 19 patients showed at least a slight improvement (0-25%). A pronounced improvement of 36.8% was recorded in 5.3% of patients, moderate in 31.6% of cases, and minimal improvement in 26.3% of patients. 12 out of 19 patients were satisfied with the treatment results. In addition, PRP was used to treat striae in combination with ultrasound therapy after plasma fractional

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radiofrequency therapy [10]. Ultrasound was used to improve the transepidermal penetration of PRP every 2 weeks for 8 weeks. It was established that the average width of the widest striae decreased from 0.75 to 0.27 mm. In a total, 71.9% of patients reported good or very good results. In addition, a histological examination of the samples obtained by biopsy revealed an increased density of collagen and elastic fibers of the dermis, although quantitative data are not given in the publication [10].

Thus, an analysis of the literature shows that scarring of the skin has a multifactorial etiology, and requires a comprehensive approach to treatment.

# CONCLUSION

At present, many surgical, conservative methods, including medical and physiotherapeutic ones, of treating cicatricialskin changeshave been offered. However, these approaches do not always allow achieving the desired results. The introduction of platelet rich plasma into the area of atrophic skin scars after acne promotes a good cosmetic effect and is accompanied by a minimum number of complications. Reducing the number of scars after platelet rich plasma therapy allows the use of this drug as monotherapy and in combination with surgical methods of treatment.

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# INTERDISCIPLINARITY IN COMPLEX THERAPEUTIC APPROACH OF PSYCHIATRIC PATIENTS WITH DYSFUNCTIONAL SYNDROME OF THE STOMATOGNATED SYSTEM

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**ABSTRACT** — Diseases in the maxillofacial sphere and especially the dysfunctional syndrome of the stomatognathic system have many connections and interferences with the psychiatric pathology and, last, but not least, the appearance and aesthetics have an impact on social life and success. On the other hand, there has been a significant increase in the use of antipsychotics in recent decades. The negative impact on oral health has also increased. The ability to diagnose mental illness is essential due to the social stigma associated with these disorders and a correct differential diagnose as well. This paper describes an oro-dental rehabilitation in one case, psychiatrically treated, with mandibular and maxilla missing teeth and dental destruction and prosthetic damage, temporomandibular disorders pathology due to the disbalanced occlusion and depression and anxiety, with specific medication, fixed and removable hybrid prosthesis and intra oral complex rehabilitation.

**KEYWORDS** — fixed dental prosthesis, edentation, depression, psychiatric treatment, oro-dental rehabilitation, temporomandibular disorders (TMD).

### INTRODUCTION

In general, for the therapeutic solution of stomatognathic dysfunctions, various and multiple means of treatment are used, which can be grouped in a prophylactic and a curative orientation.

In this phase, the prophylaxis of the dysfunctions of the stomatognathic system means in fact the prophylaxis of the dento-maxillary anomalies, of the anomalies of the muscular engrame, of the tics and vicious habits and the orthodontic adjustment must be subordinated to the gnathological functional principles (De Hert et al., 2011;Haddad & Sharma, 2007).

Most of the time, the omission of important notions of gnathology makes the orthodontic treatment to be carried out sometimes on anatomical-geometrizing or anthropological bases, forcing the growth and development factors not in functional senses, but to correspond to morphological, static patterns. Exemplification from the literature (Burlui, 2002), from this point of view, includes some morpho-geometric principles that dogmatically dominate orthodontics: mandibular incisors to make 90° with the mandibular plane and 65° with the Frankfurt plane (Tweed), the orientation of the first molars, so that the line passing through the disto-vestibular and mesio-lingual cusps meets the canine on the opposite side (Rickets) etc (Lupu, Ignat, Paduraru et al, 2016; Lupu, Ignat, Ciobotariu et al, 2016). But the application in any clinical case of these formulas and the ignoring of the functional balance that is created at the level of the stomatognathic system means in fact the activation of other points generating stomatognathic dysfunctions (Woods, 2003; Feldman et al, 2004; Tripathi, 2005; Miodownik et al, 2011; Swager & Morgan, 2011).

# CLINICAL CASE PRESENTATION

The patient B.G., 65 years old, female, retired. Father's history: none. Mother's history: Alzheimer's disease.

Reasons why the patient went to the dentist: the patient came to us to redo the dial bridge 3 and restore the functions of the stomatognathic system.

First, the anamnesis procedures, the medication taken, the clinical examination of the joint and muscles in the affected temporomandibular area where there was pain (Fig.1). Then, the paraclinical examinations, CAT scan, orthopantomography and the study model (Fig. 2) were established.

We can notice the working stages at the dental periodontal level and the adaptation of the fixed works and the occlusal readjustment (Fig. 3).



Fig. 1. Clinical examination: palpation and inspection of temporomandibular joint and the symmetry aspect of the face for clinical diagnose of dysfunctional syndrome of the stomatognathic system.



Fig. 2. Paraclinical examination model study/Orthopantomographic images and TMJ CAT scan





Fig. 3. Pro-prosthetic preparation (Pre-prosthetic preparation): preparationfor preprosthetic steps management of case treatment including plastic coronal reconstruction, endodontic treatment gingivectomyand preparation for uni-dental prosthesis

# DISCUSSION

From the point of view of plastic reconstructions, the restoration of the coronary volume in sub-occlu-

sion, although it protects the obturation or the walls of the cavity from occlusal forces, limits the support area, overloads the other contact areas, realizing the

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Fig. 4. Adaptation and finale images of rehabilitation treatment

conditions of a dysfunction of the whole system. For this reason, whenever the correct occlusal relief cannot be reconstituted by plastic fillings, it is good to apply inlay-onlay therapy, according to Black I, II, III, IV classes, or observing the principles of preparations and the retention means, or by means of the crowns of the cover for the purposes of a correct restoration of the cusps and the support areas. We also mention the fact that all coronary filling materials have a lower abrasion resistance than enamel, reason for which their occlusal face depreciates shortly after application favouring the overload of other teeth and the extrusion of antagonists. We can mention that the material of the crown components, especially metal-ceramic coatings must be in accordance with the principles of realization as iatrogenesis can be created due to abrasion and fracture that can occur in case of defective and uncorrected occlusion.

Periodontal therapy can prevent the occurrence of dysfunctions of the stomatognathic system by restoring periodontal health, reducing/disappearing mobility, limiting the phenomena of dental migration and removing traumatic inclined planes.

The reduction of parafunctions, of vicious habits prevents the installation of occlusal, periodontal, articular, muscular changes that can sometimes be accentuated, taking serious and irreversible forms.

The diagnosis of altered functions must be followed carefully, followed by phonetic re-education, mastication, swallowing, and sometimes mimicry (sigmatism, unilateral mastication, swallowing by interposing the tongue between arches, orofacial dyskinesia).

Following surgery, sequelae appear that affect the main functions of the stomatognathic system. Limiting surgical sequelae, practicing conservative surgery, functional re-educationafter surgery, are some of the objectives of surgical prophylaxis of stomatognathic system dysfunctions.

Prosthetic treatment finds its role in the prophylaxis of stomatognathic dysfunctions by fixed prosthetic restorations, such as metal-ceramic or all-ceramic joint prosthesis that prevent the migration of teeth to the edentulous spaces, reconstituting the occlusion surfaces of the maxillary and mandibular arches shortened or interrupted by oedema. By prosthetic restoration of the edentulous arches, the occlusal tampon is reconstituted, which takes over the masticatory and swallowing forces, relieving the temporo-mandibular joint. The masticatory force increases, and, at the same time, the masticatory efficiencyincreases, ensuring the stomatognathic system the conditions of an optimal morphological framework for the development of functions and thus a normal trophicity of the tissues that enter its composition (Scarff& Casey, 2011; Tani et al. 2012).

The curative therapy itself- the treatment of the dysfunctions of the stomatognathic system aims for the mental rebalancing, the muscular relaxation, the treatment of the pain, the mandibula-cranial repositioning, the occlusal-articular rebalancing, using medicinal, physiotherapeutic (Grewal et al, 2014) prosthetic methods. Within this complex therapy it is necessary a staging of the treatment phases, which will take place over more or less long periods, depending on the severity of the clinical case, for complete recovery surgical therapy is sometimes inevitable. In the same time, the necessity of a good and complete recovery can avoid the forensic aspects claims (Radu&Bulgaru Iliescu, 2016, Radu et al, 2017).

The treatment of dysfunctions of the stomatognathic system includes a complex of therapeutic, medicinal, and non-drug methods (prosthetic, physiotherapeutic, surgical), whose action is intricate, so that the therapy of pain, for example, from antirheumatic, muscle relaxation and mandibular repositioning cannot be precisely delimited.

Mental rebalancing of older-adult patients with stomatognathic dysfunction can be done by psychotherapeutic and medicinal means (Burlea et al, 2010; Carausu et al, 2017).

Psychic irritation factors that lead to the maintenance of conflict states in social microclimatestriggered the dysfunctional syndrome of the stomatognathic system (Ameida et al, Munech&Hamer, 2010. Dobri et al, 2020, Damian et al, 2017).

In psychic rebalancing therapy, drugs (medications) with hypnotic, sedative, tranquilizing action can be used. From this point of view, hypnotic sedatives are of great clinical utility, whose administration leads to a decrease in the activity of some areas of cortical excitation.

The mechanism of action aims to inhibit the activity of the motor neuronal chain, starting with the cortical motor neuron and ending with the neuromuscular junction.

To depress the activity of cortical motor neurons, some medicinal substances described in psychic rebalancing therapy act: sedative hypnotics, minor tranquilizers, major tranquilizers. In addition to the above, for the purpose of a short-term motor inhibition, which would interrupt the reflex circuits, general anaesthesia can be used in the services with appropriate equipment.

Minor tranquilizers such as meprobamate, diazepam, oxazepam have also shown muscle relaxant properties. Through their action on the limbic system, they reduce psycho-emotional lability and anxiety (Damian et al, 2017).

Thus, we can mention from the literature that meprobamate is indicated for its double effect of sedation and relaxation, in cases of bruxism. Due to its mild analgesic action, which is added to the sedative, tranquilizing and relaxing ones, meprobamate is a drug with good effects in stomatognathic dysfunctions. Diazepam is administered 2 mg three times a day, increasing the dose to 20-40 mg/day in 3-4 doses. Dose increases for both drugs can be made depending on the patient's tolerance and the severity of the case. Medazepam is a tranquilizer with a very mild sedative action, which makes it recommended for daytime use.

Medication with psychotropic drugs (sedatives, tranquilizers, anxiolytics) does not actually suppress the determining factor, but rather one of the predisposing factors. Psychotropics have the disadvantage that they keep the patient in a "psychiatric" environment that create the conditions for the installation of a drug addiction. In addition, a prolonged administration can have the following side effects: hypoxia, wakefulness disorders, side effects.

Analgesic therapy is always used concomitantly with psychotropic therapy that potentiates the action of these drugs. The treatment of pain with analgesics is only a palliative therapy because it does not suppress the cause, but only diminishes the effect, so that, after the period of action of the analgesic drug, the pain reappears. However, analgesic therapy has a recognized importance in the treatment of dysfunctions of the stomatognathic system, it being necessary to restore the patient's mental comfort, as well as to help him go through difficult periods until the cause is suppressed.

In the treatment of pain in dysfunctional syndromes of the stomatognathic system, the indication of choice is given by antirheumatic analgesics, which also have antipyretic side effects.

Subsequently, physiotherapy and kinesitherapy (movement therapy, gymnastics) are performed, they are more and more frequently used in the treatment of stomatognathic system dysfunctions (Burlui, 2002; Checherita et al, 2009;Lupu et al 2015) to obtain a more pronounced muscle relaxation, to educate normal mandibular movement patterns, to ensure a normal joint functionality, removal of tics and parafunctions. Nevertheless, an accurate and complete therapy in such cases should look at all times to the ethical and legal aspects which are involved (Toader et al, 2017).

As we have exemplified in this case a patient with mental disorders, joint damage, rheumatism, hypertension, which occurred after the related medication in the sphere of psychiatric stomatognathic rehabilitation (Tatarciuc&Panaite, 2001; Morley, 2002; Checherita et al, 2017; Zegan et al, 2017; Zegan et al, 2017; Zegan et al, 2018; Cristache et al, 2019; Zegan et al, 2019; Radulescu et al, 2020), having a dissatisfaction due to the impossibility of achieving functionality but also aesthetics at stomatognathic level.

### CONCLUSION

As shown by the rehabilitation of the patients in the study, we can conclude that drug therapy was necessary to rebalance the mental and muscular level. This is followed by the stabilization of the occlusion, providing geometry to rearrange the clinical parameters, and restoring functionality of the stomatognathic system. The complex treatment applied provided wellbeing and comfort and contributed to the increase of the quality of life and the level of acceptance of the patients.

All authors have the same scientific contribution.

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# ASSESSMENT OF REGULATORY AND REPARATIVE OSTEOGENESIS IN AUGMENTATION AREA BASED ON ORAL FLUID METABOLIC PARAMETERS

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ABSTRACT — Cases of serious atrophy of the alveolar processes occurring during dental implantation could be restored saving the respective bone volume. There is also a need for postoperative monitoring of the physiological osteogenesis effectiveness in the peri-implant zone. Our paper offers an assessment of regulatory and reparative osteogenesis in the augmentation zone subject to the oral fluid (OF) metabolic parameters obtained from 59 patients. Biochemical methods for oral fluid examination are noninvasive approaches that allow identifying pathologies at the tissue and cellular level. The proposed metabolic indices of collagen C-telopeptide, osteocalcin, alkaline phosphatase (AP) and parathyroid hormone helped identify augmentation zone of disturbed reparation in 6.8% of patients, whereas the worst complications were to be observed one month after the implantation. Through this period, however, orthopantogram featured no pathological change. Given that, the biochemical markers of bone tissue remodeling are employed for early identification and developing a forecast regarding treatment effectiveness.

**KEYWORDS** — oral fluid, metabolic parameters, augmentation, dental implantation.

## INTRODUCTION

Osseointegration of implants often has to be performed under significant atrophy of the alveolar processes [1-5]. This is most common in the upper jaw lateral parts at the maxillary sinuses. The lack of bone volume prevents implants from being placed properly and, as a result, there arises a need for bone-plastic materials to be involved, as well as ways to be found for of bone tissue directed regeneration [6]. Osseointegration in the augmentation zone should be followed, which is to done to exclude complications [7, 8]. X-ray is, undoubtedly, the main choice here, yet it is important to identify early signs of any inflammation and destructive process affecting the bone and soft tissues at the peri-implant before any clinical manifestations, which, in turn, will allow starting treatment, both due and timely. Biochemical methods for studying OF belong to modern non-invasive approaches [9, 10], which offer a way to identify the pathological process at the tissue and cellular level. All this allows assessing reparative osteogenesis at the peri-implant zone.

Studying metabolic process directly in the bone tissue in vivo is a complex issue, which explains the relevance of opting for a non-invasive method that implies assessing the bone tissue status based on data obtained through oral fluid examination, thus also making indirect judgment concerning it.

Aim of study:

to assess regulatory & reparative osteogenesis in the augmentation zone based on oral fluid metabolic indicators.

## MATERIALS AND METHODS

The clinical study involved 59 people — 21 males (35.6%) and 38 females (64.4%) aged 35–60. The patients were divided into two groups. Group 1 (main group) included 42 persons (71.2%), who were at the stage of osseointegration in the augmentation zone of allogeneic demineralized lyophilized spongy bone tissue at the maxillary sinus. Group 2 (control group) included 17 (28.8%) clinically and somatically healthy individuals. The groups were comparable in terms of age, gender, and initial periodontal status. The object of the study was the oral fluid.

The study itself focused on biochemical markers of bone modeling (osteocalcin, alkaline phosphatase, parathyroid hormone) and collagen C-telopeptide a marker of bone resorption (β-Gross Laps), while the obtained data were analyzed and underwent statistical processing.

### **RESULTS AND DISCUSSION**

For the purpose of matching the metabolism of physiological osteogenesis vs the inflammation & destruction process, we set the metabolism biochemical markers indices in the control group aiming thus to identify the values of the norm criterion. In the control group, the osteocalcin content was found to be  $0.55\pm0.006$  ng/ml; alkaline phosphatase —  $26.9\pm2.68$  U/l; parathyroid hormone —  $1.89\pm0.13$  ng/ml, collagen C-telopeptide —  $0.01\pm0.02$  ng/ml. Similar indicators were examined in the main group. The analysis showed that during Week 1, metabolic parameters remained within norm in all the patients of the main group. The subsequent analysis, though, revealed that the dynamics changed within one month (Table 1). compared with the control group — in another 4 patients.

The parathyroid hormone is a polypeptide with an action aimed at an increase of calcium ions concentration and phosphates concentration in blood plasma.

# CONCLUSION

In view of the above, a comprehensive assessment of the bone metabolism parameters in the patients' OF manifest certain changes. On the one hand, an increase

<i>Table 1.</i> Oral fluid metabolic parameters dynamics in patients of the main group ( $n=42$ ) depending on the follow-up period ( $M\pm m$ )					
		Indicator			

	Number of observations	Indicator			
Time		Collagen C-telopeptide, ng/mg	Osteocalcin, ng/ml	Alkaline phosphatase, U/I	Parathyroid hormone, ng/ml
Week 1	n=42	0.01±0.003	0.56±0.006	27.0±2.45	1.89±0.14
VVEEK I	n=0	0.01±0.002	0.57±0.005	26.3±1.8*	2.01±0.17
Week 2	n=40	0.01±0.05	0.55±0.06	27.02±2.36	1.88±0.14
	n=2	0.015±0.003	0.75±0.04**	19.0±3.1**	2.35±0.12**
Week 3	n=39	0.01±0.04	0.57±0.08	28.3±1.64	1.88±0.13
	n=3	0.02±0.005**	0.82±0.04*	10.9±1.7*	2.51±0.23*
1 month	n=38	0.009±0.09*	0.56±0.09	27.3±1.73	1.86±0.12
	n=4	0.023±0.0009*	0.93±0.07*	5.7±0.4*	2.59±0.21*

*Note.* Reliability of deviation from control: \* - at p < 0.05; \*\* - p = 0.01

2 weeks following augmentation, for instance, there was a tendency observed, which featured a certain increase in collagen C-telopeptide in 2 patients; after 3 weeks, it doubled sharply in 3 patients, whereas a month later — in 4 patients. An increase in this criterion points at a destructive process in the augmentation zone, since during the resorption process, the telopeptide with the remnants of collagen molecules penetrates the oral fluid. However, the destruction cannot be judged based on one indicator only, therefore, other criteria were additionally employed to assess the osteogenesis status. The osteocalcin content was studied, and its increase in the OF, which was observed in 4 patients after 1 month, indicated a decrease in the mineralization at the area of augmentation, contributing to relative depletion of plastic resources.

The alkaline phosphatase levels in Week 1 went down slightly in one patient (0.97%), yet, 4 patients had the maximum decrease — more than 4 times down – in a month's time. The decrease in the alkaline phosphatase activity serves proof to improper conditions for osteogenesis, namely, reduced inorganic phosphate supply at the stage of bone tissue mineralization.

The parathyroid hormone dynamics from Week 1 until 1 month of observation shows it doubling — if in C-telopeptide, parathyroid hormone and osteocalcin are indicative of destructive processes in the augmentation zone, whereas on the other, a decreasing activity of the alkaline phosphatase also indicates a slower bone formation. However, against the deep metabolic disorders, there was no change detected in these patients' orthopantomograms, which indicates early signs of structural and regulatory insufficiency. In this regard, given the fact that that 4 out of 59 patients (6.8%) had a disturbed physiological osteogenesis, which reached its maximum after 1 month, it appears reasonable to assess the above-described oral fluid metabolic parameters during the said period, thus preparing to take appropriate measures.

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# MORPHOLOGICAL FEATURES OF WOUND PROCESS IN PATIENTS WITH INFLAMMATORY AND DESTRUCTIVE PERIODONTAL DISEASES IN CASE OF DIABETES MELLITUS

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ABSTRACT — The aim of this study implied comparing the morphology of periodontal tissues in patients with diabetes mellitus following the results of surgical intervention due to inflammatory and destructive periodontal diseases. We used an erbium laser and a standard surgical method. For this, histological samples obtained from 34 patients were examined. The biopsy samples in Group 1 were studied prior to the surgical treatment of inflammatory and destructive periodontal diseases. Group 2 included histological samples of patients following surgeries employing conventional surgical techniques. Group 3 included gum biopsies obtained after surgical interventions performed with the erbium laser. The research outcomes revealed that when performed in patients with diabetes mellitus complicated with diabetic microangiopathy of the gingival mucosa, surgical curettage or a standard surgical operation with a scalpel did not lead to any significant decrease in the chronic inflammatory process. The morphological picture of the gum soft tissues in patients with Type I and II diabetes mellitus, 3 months after the treatment with the erbium laser as a cutting tool, matched the usual structure of the mucous membrane with no obvious signs of inflammation.

**KEYWORDS** — destructive and inflammatory periodontal diseases, diabetes mellitus, biopsy, erbium laser, surgical stage of treatment.

# INTRODUCTION

The treatment effectiveness of inflammatory and destructive periodontal diseases still remains one of the most serious issues faced by dentistry nowadays. Periodontitis is a multifactorial disease and can be due to various reasons [1]. The effect caused by numerous internal and external factors entails a wide

range of clinical manifestations of gingivitis and periodontitis. Diabetes mellitus [DM] is a risk factor for the development of inflammatory periodontal diseases [2, 3, 37-39] and exacerbates the severity of their course, also contributing to the development of aggressive forms. DM is a systemic disease and is recognized as a risk factor behind the onset and progress of periodontitis. An important point is that periodontal diseases, apart from all, affect the pathogenesis of numerous systemic diseases, including DM [4]. DM has an adverse effect on periodontal tissues both at the level of tissue and cells [5]. Chronic hyperglycemia contributes to the disruption of the neutrophils function while stimulating the production of inflammatory mediators, and also promotes change in the periodontal vessels vascular endothelium [6, 7]. Metabolic disorders typical of DM result in numerous cellular and molecular changes in periodontal tissues. DM has a negative impact on the course of the wound process in periodontal surgery, while slowing down the healing of wounds making it recurrent. The specific features of the wound process are due to a violated tissue blood circulation in the affected areas, disturbances in all types of the body metabolism, and an anaerobic-aerobic microflora in the purulent inflammation foci. The mechanisms involve numerous factors and include the host's altered inflammatory response, the development of glycation end products, changed bone resorption and bone tissue development, as well as altered subgingival microflora [8–14]. The microbial etiology of periodontal diseases correlates with certain types of microorganisms [15]. There is a link between the types of periodontal pathogenic agents and various subgingival microbial complexes featuring the clinical parameters pertaining to the periodontium [16]. Mechanical removal of bacterial biofilms and deposits from the root surface is the most common treatment method (the "gold standard") for cases of gingivitis and periodontitis [17]. The surgical stage is an important part of the comprehensive treatment offered to cases involving periodontal issues. Gingivectomy, vestibuloplasty, periodontal pocket closed curettage, periodontal pocket open curettage, flap surgeries, frenulotomy, frenulectomy and other invasions are traditionally performed

using conventional sets of surgical instruments, namely scalpel, curettage spoons, borers, cutters, suture material, etc. However, there are a number of factors that will reduce the effectiveness of the procedure in terms of complete bacterial elimination, such as unfavorable anatomical conditions [18], invasion of periodontal pathogens into the gingival tissue [19], and related diseases such as DM. The postoperative period includes a decrease in the soft and bone tissues, as well as the occurrence of purulent-inflammatory complications and relapses. Surgical infection in the inflammation foci complicates the course of purulent diseases, slowing down the reparation. The traumatic nature of surgical interventions, postoperative complications and the need to preserve the tissues involved in the pathological process as much as possible demand new treatment options, surgical laser technologies being one of them.

As far as the cutting tool is concerned, the Er: YAG laser offers an alternative to a conventional scalpel. Laser is good when treating patients with systemic factors that can change periodontal tissues biological response through regeneration after mechanical treatment and surgery, or may contribute to the disease progression. Speaking of these modifying factors, an important role here belongs to diabetes mellitus [20, 21]. The Er: YAG lasers are used for the sanation of the dentoalveolar pocket, the elimination of periodontal pathogens, as well as for scaling and root planing 22. The effect of the Er: YAG laser is similar to that of scaling with root planing [27-30], with a more prominent reduction in the microbial population in the long term, when working with the laser [31]. The clinical effect of the laser is comparable to scaling with root planing when treating periodontitis [30-32]. The Er: YAG laser, unlike other lasers, is effective for surgery involving soft tissues and mineralized tissues. The laser ensures ablation with the required hemostasis and a decrease in the bacterial load [23]. Procedures for lengthening the clinical crown of a tooth, frenulectomy, vestibuloplasty, preparation of the bed tissues for prosthetics, removal of excess gum tissue arising through injury and the use of medications, gingivectomy or gingivotomy, are performed using powerful lasers featuring sufficient hemostasis, while the clinical procedure is more conservative [24]. Lasers shorten the procedure duration, reducing the need for flaps and sutures. Employing the Er: YAG lasers for periodontal surgery allows enhancing tissue regeneration, helps perform surgeries without any discomfort caused during or following the treatment, and with no postoperative sensitivity, pain or bleeding. Activated metabolic processes of cells and improved microcirculation after laser treatment, promotes reparative processes in the area of the surgical wound [33]. The Er: YAG

lasers allow making incisions as well as excision of the gum soft tissues and mucous membrane, yet, their use is limited to flap surgery [25]. Dental lasers have been developed using fiber optics helping gain access to periodontal pockets, which, in turn, ensures a powerful bactericidal effect. The ability of water molecules to absorb laser radiation is the factor that determines its penetration into tissues, as well as its capacity to cause thermal damage to surrounding tissues [26]. The Er: YAG laser radiation (2940 nm) makes it effective both for operating on soft tissues and on mineralized tissues. The laser energy is absorbed into the respective gum tissue, while there is no unwanted thermal damage affecting the adjacent tissues (enamel, dentin and bone). This factor makes the laser-involving procedure easy to perform, also allowing conservative and precise removal of the target tissue, especially in areas where periodontal aesthetics is something to be taken into consideration. Using the erbium laser in periodontal surgery in an outpatient setting offers a number of intraoperative and postoperative advantages, namely, reduced tissue traumatization through the surgery, high-quality hemostasis, shorter tissue regeneration time, and prevention of postoperative edema.

### Aim of study:

to carry out a comparative morphological investigation into the wound process in patients with inflammatory and destructive periodontal diseases against Type I and II diabetes mellitus following surgical intervention involving the use of both the erbium laser and a scalpel.

# MATERIALS AND METHODS

The study was conducted at the Department of General and Clinical Pathology: Pathological Anatomy, Pathological Physiology (Samara State Medical University, Russia) and in the histological laboratory of the clinic pathology department (Samara State Medical University, Russia).

The clinical data is based on what was obtained through surgical treatment offered to patients with infection purulent foci affecting periodontal tissues. The inclusion criteria for the study were chronic types of periodontitis against Type I and II diabetes mellitus. There were no differences made as to the form of diabetes mellitus, since all patients reveal identical clinical signs of the diseases. A total of 34 patients underwent surgeries and were observed subject to the indications (26 - females; 8 - males). The average age of all the patients was 56±5. The average age, gender, the nature of the purulent-necrotic process, as well as other factors were comparable in the 3 clinical groups that the patients had been broken into. The inclusion in each group was based on a voluntary and informed consent. The study meets the requirements set by the Committee for Bioethics functioning at the Samara State Medical University (of 27/01/2010).

To study the morphological features of the gum tissue, biopsy samples were taken at different stages of the surgical treatment. The entire body of samples through the study included 136 histological preparations. The morphological material was studied in 3 groups. The first — control — group, included fragments of the gingival mucosa tissues obtained from patients prior to the start of treatment. The samples in the group were examined straight after taking biopsies. The second group included biopsies of patients following surgeries performed with conventional surgical techniques using a scalpel, curette spoons, borers, cutters, etc. The sample collection as well as the study were done 7–10 days and 1–3 months after surgeries at the stages of comprehensive treatment. The third group — the one under study — had gingival mucosa tissues obtained after surgical interventions on periodontal tissues, with the Er: YAG laser (2940 nm) used (KEY Laser \* 3; KaVo, Biberach, Germany) as the cutting tool with the parameters of 120 mJ, 10 Hz, and the supply of an aqueous spray to the target tissues. The sampling and the study were carried out 7–10 days and 1–3 months following operations on periodontal tissues. The morphological studies were carried out using biopsy samples for histological analysis. The samples included soft tissue fragments of marginal periodontium (volume  $-0.2 \times 0.5$  cm; 2–4 pieces as per each observation). The samples were fixed in 10% neutral formalin and, according to the commonly accepted method, poured into paraffin blocks. Histological sections made of paraffin blocks (4–5 microns) were stained with hematoxylin and eosin, picrofuxin. The completed preparations were examined with a photomicroscope, analyzed and photographed.

This work relied on the calculation of the percentage ratio (volume density) for epithelium, connective tissue, inflammatory infiltrate on preparations stained with picrofuxin subject to the method of point count based on the Videotest-morpho image visualization software. The statistical processing of the respective data was done on the Statistica standard software package in Windows. The histometric study algorithm included carrying out the measurements themselves (12 in each histological sample), preliminary measurements with the identification of the required number of counting signs, the identification of the correction factor for the tissue shrinkage, and checking the hypothesis for the distribution normality.

# RESULTS

The first group's histological samples obtained prior to surgery, featured morphological signs of chronic inflammatory periodontal diseases in the acute stage. Microscopic examination of the bloodstream revealed signs of diabetic microangiopathy in biopsies. The capillary vessels were found to have thickened walls, which was due to puffiness and the development of protein lumpy masses. Congestion of small-size vessels also featuring signs of exudative reactions was observed against morphological changes in connective tissue and multilayer squamous epithelium. The typical polymorphocellular infiltration of connective tissue with the plasma cells to be observed, as well as diffuse lymphoid tissue, point at the inflammation chronization in the studied gingival fragments. The evidence of a high inflammatory activity can be found in leukostases and leukocyte infiltration in the areas of necrotically altered epithelium within the pathological focus. The materials contained typical signs of coarsening collagen fibers in connective tissue with a dominating cellular component, dysplasia and metaplasia of the epithelial layer. Collagen fibers, as well as vessel walls in the preparations, were observed to be of different thickness with varying tinctorial properties when stained with picrofuxin (Fig. 1).

The gingival mucosa preparations that were studied, were observed to bear features typical of dysregulation against the background of proliferative processes. The presence of a granular layer in a multilayer flat non-keratinizing epithelium meant a tendency towards keratinization. In the histological preparations, we could observe thinning the epithelial layer with smoothed papillae. A significant loss of vertical anisomorphy was to be seen in the biopsies, with a mild degree of epithelial cell dysplasia present. There were certain features of leukocyte infiltration of the basal and spinous epithelial layers with areas of intraepithelial desquamation. In addition, acanthosis with edema, a changed structure of epithelial cell contacts and vacuolization of the Malpighian layer elements were obvious in the epithelial lining (Fig. 2).

In Group 2 samples obtained one month following periodontal surgery, blood vessels with changed wall thickness, puffiness and endothelium swelling remained (Fig. 3).

Proliferative-reparative changes were observed in the preparations; fibrous changes could be seen in the connective tissue, while granulation tissue prevailed over mature collagen fibers. In the histological preparations, dysregeneration signs were to be seen in the stromal structures and epithelium. The collagen fibers in the examined fragments differed in maturity and tinctorial properties (Fig. 4).



*Fig. 1.* Deposition of lumpy protein masses in an arteriole with a swollen wall. Staining: picrofuxin × 900



Fig. 2. Acanthosis symptoms in the multilayered flat nonkeratinizing epithelium, inflammatory infiltration in the underlying tissue. Staining: hematoxylin and eosin  $\times$  100



**Fig. 3.** Gum histological sample, 1 month following the periodontal surgery. Multilayered squamous epithelium, granulation tissue, stroma edema, small vessels with swelling endothelium and thickened wall. Staining: picrofuxin × 300

The third group of biopsies obtained 7–10 days following the surgery using laser technology, revealed no obvious signs of diabetic microangiopathy affecting small arterial vessels. The histological preparations showed no signs of acute inflammation manifested as leukocyte infiltration or necrotic component. Unlike the other two groups, the multilayered flat non-keratinizing epithelium had a normal structure of the oral mucosa. The spinous layer was thicker while acanthotic papillae appeared elongated. The connective tissue stroma had a fibrous component prevailing, which had varying degrees of collagen fibers maturity. The signs of dysregeneration persisted, evidence to that being thin collagen fibers and lumps of collagen surrounded by developed fibrous tissue. The preparations in the said group taken 3 months later, had mature fibrous connective tissue and multilayer flat non-keratinizing epithelium with no signs of inflammation. The examined fragments had a structure that virtually never differed from the normal structure of the gingival mucosa (Fig. 5).

The examined morphological material showed no signs of diabetic microangiopathy, the blood vessels had differentiated walls, with neither inclusions nor thickening. The epithelium was located above the connective tissue base. The epithelial lining thickness, the size and location of the papillae featured no disturbance. The spinous layer had no signs of pathology. Epithelial cells revealed no signs typical of metaplasia, dysplasia, necrobiotic or dystrophic issues. Mature or tough collagen fibers prevailed in the connective tissue. Single focal perivascular lymphocytic infiltrates could be observed. No sign of inflammatory infiltration was odentified (Fig. 6).

Histometric examination suggests signs of subsiding inflammation. The laser technologies, when used for the treatment of tissues at the operated area, left certain signs of reparation, with developing connective tissue and the restoration of the epithelial lining structure. As soon as seven days following the surgery, the area of the pathological periodontal focus where the erbium laser was used, showed a significant increase in the volume density of the vascular component and connective tissue against a decrease in the inflammatory infiltrate volume density. The volume density of the epithelium revealed basically no change. The epithelial lining thickness was not affected by any decrease in the edema or a lower number of intraepithelial leukocytes and microvessels (Table 1).

## DISCUSSION

Inflammation affecting periodontal tissues reveals irreversible loss of the tooth-supporting structures, including the gum connective tissue fibers, the



*Fig. 4.* Connective tissue proliferation. Collagen fibers featuring various thicknesses and tinctorial properties. Staining: picrofuxin  $\times$  100



*Fig. 5.* 3 *m* following laser treatment. Normal structure of the mucous membrane. Staining: hematoxylin and eosin × 300



**Fig. 6.** 3 m following laser treatment. Multilayered flat non-keratinizing epithelium with no signs of acanthosis; moderate fibrosis seen in the underlying tissue. Staining: hematoxylin and eosin × 100

**Table 1.** Tissue volume density ratio vs. inflammatory infiltrate in gum biopsies, patients with Type I and II diabetes mellitus through surgical curettage and laser treatment following treatment for inflammatory and destructive periodontal diseases,  $(M\pm m)$ 

	Volume density index (%)		
Observation term	Connective tissue	Epithelium	
Surgery with a scalpel	17.3 ± 3.8	$42.3\pm3.6$	
1 month following the surgery (scalpel)	29.7 ± 2.8	$36.4 \pm 4.1$	
7–10 days following treatment with laser	56.1* ± 3.6	$27.5^{*} \pm 2.3$	
1–3 months following treatment with laser	66.2*±3.7	24.5* ± 2.6	

Note: \* — statistically meaningful difference, p<0.05

periodontal ligament and the alveolar bone. Local irreversible tissue destruction leads eventually to partial or complete loss of teeth [34, 35]. The link between DM and periodontitis is actually bilateral [8], which means there is a need for ways to improve the treatment effectiveness when dealing with these diseases. The treatment outcomes for periodontitis in patients with DM, when low-power lasers are used, are available [36]. Employing the Er: YAG laser (2940 nm) to treat inflammatory periodontitis issues against DM allows obtaining outcomes that differ from those offered by standard approaches. The research described above proves the effectiveness of the Er: YAG laser (2940 nm) in periodontal surgery, when used as a cutting tool, as well as a means of tissue biostimulation. The Er: YAG laser irradiation has proved to be a comfortable choice for patients, which offers a high therapeutic effect causing no thermal damage to the surrounding tissues, unlike other lasers. Compared with standard methods, this type of laser is more conservative and gentler when it comes to periodontal tissues, while there have been no side effects reported when assessing the safety of using a laser at respectively specified wavelength and power.

## CONCLUSION

Taking into account the morphological features pertaining to periodontal tissues in patients with Type I and type II diabetes mellitus after surgical intervention the use of the erbium laser proves to have certain advantage over conventional scalpels. Using a dental laser to treat complex treatment of inflammatory and destructive periodontal diseases entailed no signs of diabetic microangiopathy in histological samples. The morphological presentation of the gum tissues reflected the common structure of the mucous membrane with restoring epithelial-stromal ration and an increase in fibrosis without any distinct signs of inflammation. The use of the erbium laser as a cutting tool offers a completely new potential for the rehabilitation of chronic infection foci in the oral cavity, and ultimately improves the quality of life of patients suffering from diabetes mellitus.

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# METHODS OF POSITIONING CYLINDRICAL DENTAL IMPLANTS: A LITERATURE REVIEW

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ABSTRACT — Advanced digital technologies and respective software offer dentistry much wider opportunities. Computed tomography, for one, is becoming more and more affordable and almost every dental tomograph has the necessary software installed for dental manipulation 3D planning. Dental implantation in Russia has seen significant development allowing dental implants to be installed even with scarce bone tissue. The purpose of this paper is to offer a review of various methods that can be employed to plan the installation of a dental implant as well as the preparation of surgical templates. Dentistry has always had a close connection with other fields of science and industry, attracting a large number of innovations. Here, we have collected data showing how the treatment procedure is changing through integration of computed diagnostics technologies (CT) and manufacturing (CAD/CAM) technologies. Consequently, we gain access to more efficient and less traumatic dental implantation planning systems, all this being based on accurate data and computer calculations that minimize any potentially negative technological or human factor.

**KEYWORDS** — dental implantation, CAD/CAM technologies, navigation templates.

## INTRODUCTION

Advanced digital technologies and respective software open new and much wider opportunities before dentistry [1–3,10,40–42]. Computed tomography, for instance, is becoming more and more affordable. Literally every dental tomograph has the required software for 3D planning of dental manipulations. Dental implantation in our country has got significant development, allowing dental implants to be installed even with scarce bone tissue [16, 18, 63]. Dental implantation in our country has got a significant progress impetus. Dental implants now can be installed even with insufficient bone tissue. Such interventions benefit greatly from using guiding templates. Their application technology implies preliminary planning of the parameters and the location of dental implants, setting their number and axial position [4-6, 14, 17, 51, 62]. After that, a surgical template is made in the laboratory, which must be fixed securely on the prosthetic bed and have guides for surgical cutters, which are used to prepare the implantation bed [19, 20, 49, 52]. This article offers an overview of various methods of dental implant installation planning, as well as the manufacture of surgical templates. Preliminary planning of the dental implant is carried out on plaster models or relies on X-ray examination data [7-9, 11, 21-23].

# METHOD OF BIOMETRIC PLANNING ON PLASTER MODELS

Once the impression is taken, the prosthetic bed topography is transferred to the plaster model. The thickness of the mucous membrane at the dental implant is detected through the sound method, which involves a sterile probe with a marker to be used. As a rule, 5 points are used for probing at each site: the vestibular and oral parts of the alveolar process base at the level of 2/3 of the alveolar process height and along its apex. A so-called fabric map is created, which helps to transfer the data to the plaster model. The model is cut out, with respective points marked on each surface that was involved in mapping. Afterwards, they are connected, which results in the topography of the bone tissue at the implantation area.

# COMPUTER SYSTEMS FOR X-RAY DATA ANALYSIS

Computer analysis facilitates the identification process: the number of implants; the shape and size (length and diameter) of the implants; possible issues in the bone tissue around the implants; possible need for bone grafting. Nowadays, there are many computer systems available on the market, while all of them could be divided into: A) systems relying on radiographic data; B) systems using computed tomography data; C) systems employing computed tomography data, with possible online computer navigation; D) systems using computed tomography data with possible manufacture of a surgical template [24, 25, 43–45].

## SYSTEMS EMPLOYING X-RAY DATA

There are several software products available on the market, which are some type of specialized 2D image graphic editors based on orthopantomograms [26–28, 54, 59, 61]. These allow certain accuracy in terms of identifying the implant topography, the implant shape, implant (diameter and length), the need for sinus lifting and methods for that, as well as the type and shape of future restoration [30].

However, due to the 2D nature of the data offered for analysis, as well as given possible distortions of up to 10% vertically and up to 20% horizontally, when analyzing orthopantomograms, the systems in question appear to have serious limitations if used in complex anatomical circumstance [12, 15, 29].

# SYSTEMS EMPLOYING COMPUTED TOMOGRAPHY DATA

Computed tomography is 40–50 times more sensitive, if compared to the conventional X-ray examination, since it features a better visualization for the difference in the object density, thus allowing designing its 3D shape with accurate dimension details [46–48]. These features will help plan the installation of a dental implant in 3D space, which virtually guarantees the exact topographic integration of the implant into the bone. Therefore, the recent years have witnessed a number of software products coming to the market that, since based on CT data, allow virtual installation of 3D implant samples in model of jaw bones, at the same taking into account the height and width of the respective bone tissue, as well as the mandibular nerve position [31–33, 55, 56].

# SYSTEMS EMPLOYING COMPUTED TOMOGRAPHY DATA WITH POSSIBLE ONLINE COMPUTER NAVIGATION

### *I. Equipped with infrared sensors*

Video surgery (surgery under visual monitoring) is something to be seen in many areas of medicine. This system incorporates the GPS (Global Positioning System) principle. Such systems allow not only CT planning of dental implant installation, yet also monitoring it in the real time mode on the screen, which displays the projection of the tip and the working surgical cutter involved in preparing the implant bed, in relation to the jaw tissue bone, which, in turns is a way to help control the entire process of preparing the bone bed through the surgery [7, 8, 34, 35, 52]. The principle of such system operation relies on the technical features of the equipment used:

Example of the dental navigation system. Fig. 1, 2.

1. The system – the emitter tip. Next to the patient's chair, there is a unit including a monitor and a LapDoc platform, with an infrared stereo camera on it, equipped with optical diode sensors. The handle of the nozzle is equipped with three infrared emitters. The position of the tip is calculated in relation to the jaw.

2. The system — the alveolar process radiator. During the operation, the system identifies the jaw position with an acrylic template attached to the teeth or the alveolar bone. The bezel has a U-shaped register with 10–12 ceramic (titanium) balls (diameter — 3 mm). The optical system offers an accuracy of ab. 1 mm. The downside of this system is that there must be free passage of the infrared beam with no refraction from the emitter to the sensors. In case of the beam refraction the system will issue a notification (signal) [36].

#### II. Equipped with ultrasound sensors

One of the latest developments in the area of online navigation. The pilot plant was developed by the European Institute of Implant Robotics (Institut Europeen de Robotique Implantaire / France), its difference from the two above-mentioned systems being the type of sensors it relies on — not infrared yet ultrasonic. The tip is positioned with an accuracy of 0.3 mm, unlike the conventional infrared systems, which ensure a good static calibration of 0.1 mm, yet feature poorer dynamics — 0.6-1 mm. The system described herein provides a static accuracy of 0.05 mm, the dynamic accuracy reaching 0.3 mm.

The methods employed to manufacture surgical templates can be broken into two main groups: templates manufactured following the classical laboratory technique, and those manufactured with computer systems.

### Manufactured based on the classical laboratory technique.

In dental implantology, there are various options used to manufacture surgical templates by hand.

1. The simplest way is to make an analogue of a removable prosthesis in a dental laboratory, with a partial overlap on the teeth remaining on the jaw on its base, thus ensuring its effective fixation through the surgery. Artificial teeth in such sort of a prosthesis give an understanding of the localization for further dental implant installation.

2. A modified version of the method described above, which allows simultaneously planning the dental implant location using CT data, which relies on the fact that either a radiopaque substance is to be applied to the template artificial teeth prior to computed tomography, or the artificial teeth themselves are to be made with such substances added. In this case, a CT



**Fig. 1.** The alveolar process bed preparation to install the implant; a — the reference pin; b — the bone cutter is matched against the reference pin; c — the physio dispenser tip; d — the laser emitter; e — the occlusive stabilizer; f — the craneostat; g — the extra notification system displaying the zero (reference) angular position of the bone cutter



**Fig. 2.** The positioning and installation of the dental implant in the alveolar ridge. a — the physio dispenser tip; b — the laser emitter; c — the craniostat; d — the extra video broadcasting system

scan with an X-ray model of this type is done in the patient's oral cavity, through which the doctor will obtain not a 3D image of the jaw anatomical structures alone, but the spatial position of the planned artificial teeth, which will help planning the installation of dental implants.

3. Ez Stent Technology (Applied Dental Inc.). When dealing with defects embedded in the dentition, the technology in question allows an easier creating of a surgical template using a thermoplastic billet with a titanium casing in the center. The template, if placed for 1 minute in a rubber cup with 60° C–hot water, becomes transparent as well as gains elasticity. In this condition, it is placed on the model at the implant, and the two adjacent teeth in the defect area get compressed. As template loses its transparency, it means it is gaining its rigidity back, along with and readiness for use.

4. Making a template by thermo-forming takes first marking the plaster model with spots pointing at the areas for the subsequent implantation, and these are the points where the teeth are to be modeled with wax or glued together as taken from a set.

Then, a vacuum forming machine (e.g., Plastvac, Erkoform, etc.) is to be used to press a sheet of thermoplastic material (thickness — 3 mm) against the surface of the cast model, while maintaining a specified temperature mode, under vacuum conditions. After cooling, the mold is to undergo processing. Wax teeth are removed with a jet of water, and the model is to get perforated at the sites for implants to be installed. Following that, the entire vestibular part of the template is cut out at the next implant, nearly coming to the center of the alveolar ridge.

# DENTAL IMPLANTATION PLANNING SYSTEMS EMPLOYING COMPUTER TOMOGRAPHY DATA WITH POSSIBLE MANUFACTURING OF A SURGICAL TEMPLATE (CAD/CAM TECHNOLOGY)

The advance of computer control systems has created grounds allowing the introduction of CAD/ CAM into dental practice, which in most cases makes it possible to do without human involvement through the orthopedic devices production stage.

In dentistry, this technology has penetrated into the production of non-removable orthopedic devices, and now is going on to be used further — in the production of surgical templates as well as in dental implantology [37, 38, 60].

There are two types of CAM systems used in dental implantology nowadays:

I. Prototyping. II. Sequential deformation of a thermoplastic CAM.

#### I. Prototyping.

Compared to other methods (making models of foam, wood, wax by hand or on CNC machines), which had been common until the mid-1980s, the advance of rapid prototyping systems proved to be a technological revolution.

Prototyping is a new technology that is progressing actively in R&D (design and production). It allows obtaining physical parts and models with no actual tool production, and implies converting data from the CAD system, while having previously obtained 3D drawings and projects.

There are three types of surgical templates used for dental implants:

1. A template relying on a bone. The e-type of the surgical template is modeled using a 3D CT model, created on a stereolithographic device.

2. A template based on adjacent teeth in the defect area (a mandatory condition is the availability of two adjacent teeth on each side of the defect).

3. A template relying on the mucosa.

VIP (Implant Logic SYSTEMS), USA

Impressions should be obtained both with a prosthesis (temporary restoration) and without one. The position of the central occlusion (the upper jaw ratio) is to be identified, too. After that, the doctor contacts the Implant Logic Systems or gets a request form from the company's website. Upon completing the form, it is to be sent, along with the template, to the company to get the radiographic templates produced, and after surgical models are made, CT diagnostics is performed, with the respective data recorded in DICOM 3 format. Using the software, the doctor carries out the dent planning. The planning information is then sent to the company's laboratory, where a surgical template is made [13, 19, 50, 53, 57].

The company supplies the types of surgical templates for the doctor to choose from:

1. The basic Compu-Guide<sup>®</sup> Template. This template allows the cutter to move directional through drilling. It contains 2 mm sleeves with precise position, angle and vertical level to ensure due surgical protocol.

2. The Compu-GUIDE" ADVANCED clamp relies on a set of replaceable bushings that can be replaced stage by stage, depending on the perforation level, which allows full control of osteotomy with the surgical template.

Besides, a removable prosthesis (Compu-Temp <sup>™</sup>) can also be prepared, where the surgical cases are located to ensure the fixation of the temporary structure following implantation.

CAD Implant (CAD Implant, Inc.), USA. The company has been using this technology since 1994. The destination of the model is mucous membrane. First, computed tomography with radiographic templates is done, which takes a registration cube (CAD Implant Registration Cube). Using special software, the doctor models a surgical template, the e-version of which is sent to the CAD Implant, Inc., where it is manufactured through stereolithography and computer milling [24, 58].

Implant Master (iDent), USA, Israel

The company's promotion claims that iDent Imaging is a dynamic visualization technology that simplifies, as well as improves qualitatively, the accuracy of planning and installing dental implants. However, nothing is mentioned regarding how this effect is ensured. There is a computed tomogram with an X-ray template done; the doctor analyzes the anatomical area and plans installation of the next dental implant. The planning data from the Implant Master is delivered to the iDent service center, where the surgical template is produced digitally.

Planning involves the Procera Software Planning — software based on a 3D analysis of a dental implant. The software helps identify the optimal topography of implants, in view of the anatomical status, as well as the orthopedic and aesthetic requirements. In case of full adentia, three horizontal locking pins (d = 1.5 mm) ensure the surgical template is fixed reliably during the surgery. In case adentia is of partial nature, fewer pins are to be used, whereas cases of only tooth missing require fixture relying on the neighboring teeth.

The requirements for the surgical template are as follows: thickness — at least 2.5–3.0 mm. Enhanced strength can be achieved through reinforcement.

#### II. Sequential thermoplastic CAM-deformation

Used currently only by the TactileTech company in the ILS (Implant Location System). This system features a number of functions that make it different from others. Apart from computed tomography data, it can be used to detect the degree of the bone tissue mechanical elasticity in the alveolar process in at the implantation area. For this purpose, a special frame is used, which is fixed on the alveolar process. Further, a matrix with microneedles is installed on it, while the needles penetrate into the gum tissue up until they come into contact with the bone. The elasticity coefficient is determined by the vestibular and oral surfaces. Further on, the information is transmitted to the analytical unit, where a dental implant installation is planned relying on the data from the elasticity coefficient and computed tomography. The information is then referred to the CAM device. There is a hollow

plastic tube installed in a special cylinder bed, while the other part of it is fixed on a rod, which is involved in the following deformation under the effect of respective temperature conditions [22, 35, 36].

Thermal sequential deformation of the pipes is followed with them installed in a block that is mounted in a frame system.

## CONCLUSION

Dentistry has always enjoyed close connection with other fields of research and production, attracting a large number of advanced innovations and developments. This article offers an analytical view on the treatment changes, which are due to the integration of computed diagnostics (CT) and manufacturing (CAD/CAM) technologies. Given that, there are more efficient and less traumatic dental implantation planning systems available, which are based on accurate data and computer calculations, which, in turn, minimizes potential effect of technological or human factors.

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#### METHODS TO INCREASE THE TREATMENT EFFECTIVNESS FOR INFLAMMATORY DISEASES OF PERIODONTIUM IN CHILDREN Received 23 September 2021; Received 13 September 2021; Received 19 October 2021; Accepted 29 October 2021;

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**ABSTRACT** — The study enrolled schoolchildren diagnosed with chronic catarrhal gingivitis (CCG) and mild form of chronic periodontitis (MFCP). Total 103 patients included 67 patients with chronic catarrhal gingivitis and 36 patients with mild form of chronic periodontitis. The results of laboratory studies showed that in the process of conduction of the therapeutic and preventive measures during the use of a Loroben solution the functional activity of the local immune factors was significantly recovered. During complex treatment of these patients the use of a Loroben solution provided more significant improvement of the indices than the use of the chlorhexidine bigluconate. Loroben solution improves trophic factors of the periodontal tissues, contributes to the elimination of gum bleeding and inflammation, thus, leading to a positive treatment outcome.

**KEYWORDS** — catarrhal gingivitis, chronic periodontitis, schoolchildren, Loroben.

# INTRODUCTION

Periodontal diseases occupy a special place among the main dental pathologies. The history of the study of the etiopathogenesis of these diseases is very ancient. Over the past centuries, numerous theories and classifications of periodontal diseases have been suggested and many researches have been carried out. Epidemiological studies show that the observation of periodontal diseases is increasing in the 21<sup>st</sup> century as well. According to the World Health Organization, these values are close to 80–92% [5].

Along with the confirmation of the wide spread of periodontal diseases among children and adults, modern epidemiological researches allow to prove the observation of these diseases at an earlier ages too [1, 2, 8, 9]. The etiology and pathogenesis of periodontal diseases, their clinical course, pathological changes should be used under the supervision of clinical, paraclinical and laboratory studies. Despite the successful results of numerous researches, this problem still remains relevant and creates prospects for applications of new diagnostic and practical methods.

Therefore, one of the main aims of modern dentistry is to study methods to enhance the effectiveness of treatment for inflammatory periodontal diseases [6, 7, 3].

#### *The aim of the study*

was to evaluate of the effectiveness of the use of a Loroben antiseptic solution, including a combination of effective components.

## MATERIAL AND METHODS

The study involved 103 patients with a diagnosis of chronic catarrhal gingivitis (CCG) and mild form of chronic periodontitis (MFCP). Of these, 67 people were patients with chronic catarrhal gingivitis and 36 patients with mild form of chronic periodontitis.

The patients were divided into 3 study groups:

Group I — without prescribing any therapeutic measures, professional hygiene of the oral cavity was carried out;

Group II — after professional hygiene of the oral cavity, a solution of chlorhexidine bigluconate was used;

Group III — after professional hygiene of the oral cavity, the Loroben solution was used.

The studies were carried out on the  $3^{rd}$ ,  $5^{th}$ ,  $7^{th}$  days of the treatment process, as well as closer to the end of the treatment — on the  $14^{th}$  day. Objective dental tests were used, mathematical and statistical analyzes were carried out.

To determine the persistence of the long-term effect of therapeutic and preventive measures, the follow-up results of the clinical state of the oral cavity were carried out.

# **RESEARCH RESULTS**

Dynamic observation of changes in the oral cavity resulting from therapeutic and preventive measures showed that in patients with CCG and MFCP, after the 3rd day of using the Loroben solution, significant positive changes in the studied indices were observed.

Before the treatment in patients with CCG, the value of PMA index was 33.8±2.3, the Greene-Vermillion hygiene index (OHI-S) was 2.24±0.19, and the periodontal index (PI) was 1.69±0.12 on average and the sulcus bleeding index (SBI) averaged 1.68±0.12 (Table 1)

On the  $3^{rd}$  day, the patients' complaints of discomfort decreased, but in 9 (25.7%) patients, the observation of slight bleeding during tooth brushing continued.

Having decreased by 57.7%, the average of OHI-S index was 1.10±0.10. Having decreased by 51.2%, the PMA index was 24.2±1.6, the PI index, having decreased by 4.4%, was 2.85±0.20. During the examination, slight hyperemia and

edema of the gingival papillae, bleeding during prob-

Table 1. Dynamics of clinical indicators in patients with chronic catarrhal gingivitis before and after the use of the Loroben solution

Clinical indices	Before treatment	Treatment dynamics				
		3 day	5 day	7 day	14 day	
OHI-S	2,24±0,19	1,05±0,06	0,58±0,04	0,24±0,02	0,12±0,01	
	(1,1 – 3)	(0,4 – 2) ***	(0,1-1,3)***	(0-0,8) ***	(0-0,4) ***	
PMA,%	33,8±2,3	20,6±1,8	12,4±1,1	7,8±0,6	5,6±0,4	
	(41 – 66)	(30 – 31) ***	(8 – 21) ***	(4 – 15) ***	(2 – 12) ***	
SBI	1,68±0,12	1,07±0,09	0,65±0,05	0,26±0,01	0,10±0,01	
	(1,3 – 3,2)	(0,5 – 1,8) ***	(0,3-1,2)***	(0-0,8) ***	(0-0,4) ***	
PI	1,69±0,12	1,23±0,09	0,96±0,05	0,32±0,02	0,11±0,01	
	(1,1 – 3,5)	(0,8 – 1,6) **	(0,5 – 1,4)***	(0,1 - 0,9)***	(0 – 0,3) ***	

*Note:* Statistical significance of the difference with the measurements before treatment: \* - p < 0.05; \*\* - p < 0.01; \*\*\* - p < 0.001 (W - according to Wilcoxon).

Having decreased by 53.1%, the OHI-S index equaled to  $1.05\pm0.6$ , and the PMA index, having decreased by 39.1%, amounted to  $20.6\pm1.8\%$ . During the examination, slight hyperemia and edema of the gingival papillae were noted.

The PI index, having decreased by 27.2%, became  $1.23\pm0.09$ . Bleeding during probing was observed only in 6 patients. Having decreased by 61.7%, the SBI index was  $1.07\pm0.09$ .

Before treatment, in 14 patients diagnosed with MFCP, the average PMA index was 49.6 $\pm$ 3.8, the Greene-Vermillion hygiene index (OHI-S) averaged 2.60 $\pm$ 0.22, the periodontal index (PI) — 2, 98 $\pm$ 1.22 and the sulcus bleeding index (SBI) averaged 2.66 $\pm$ 0.21 (Table 2).

ing were noted. The SBI index, having decreased by 20.7%, averaged 2.11±0.16.

During the entire observation period, the statistics showed a slight decrease in the values of periodontal indices. PMA index decreased by 81.3% ( $9.3\pm0.8$ ), PI index — by 85.2% ( $0.44\pm0.03$ ), SBI index — by 78.9% ( $0.56\pm0.04$ ). Oral hygiene was considered good, the average OHI-S index decreased by 88.1% was  $0.31\pm0.02$ .

To determine the persistence of the effect of therapeutic and prophylactic measures in the future, the long-term results of the clinical state of the oral cavity were checked.

In children with CCG, included in the main group, after 3 months, gingival bleeding and visu-

Clinical indices	Before treatment	Treatment dynamics				
		3 day	5 day	7 day	14 day	
OHI-S	2,60±0,22	1,10±0,10	0,72±0,05	0,51±0,03	0,31±0,02	
	(1,1-3)	(0,6 – 1,8) ***	(0,4-1,4) ***	(0,2 - 1) ***	(0,1-0,6) ***	
PMA, %	66,6±5,8	34,2±2,2	16,8±1,4	11,2±1,0	9,3±0,8	
	(51 – 75)	(25 – 45) ***	(24 – 36) ***	(7 – 19) ***	(5 – 16) ***	
SBI	2,66±0,21	2,11±0,16	2,06±0,15	1,81±0,14	0,56±0,04	
	(1,4-3,8)	(1,3 – 3,2) *	(1,2-3) *	(0,8 – 2,6) **	(0,2-1,2) ***	
PI	2,98±0,22	2,85±0,20	2,33±0,18	1,93±0,14	0,44±0,03	
	(2-4,1)	(1,8-3,9)	(1,2-3,4) *	(1 – 3) ***	(0,2-1,1) ***	

Table 2. Dynamics of clinical indices in patients with mild form of chronic periodontitis before and after local use of the Loroben solution

*Note:* Statistical significance of the difference with the measurements before treatment: \* - p < 0.05; \*\* - p < 0.01; \*\*\* - p < 0.001 (W - according to Wilcoxon).

ally detectable areas of inflammation were absent. In isolated instances, the presence of these signs was due to non-compliance of oral hygiene or incomplete treatment (Table 3). 8 patients were complaining on discomfort and bleeding gums during toothbrushing. Clinical examination revealed slight hyperemia and swelling of the gingival papillae and bleeding while probing. But, on 14<sup>th</sup> day

	)bservation dynamics				
Clinical indices	After treatment	3 months	6 months	12 months	
	(n=35)	(n=28)	(n=23)	(n=17)	
PMA, %	5,6±0,4	6,8±0,6	8,2±0,7	10,8±1,0	
	(2–12)	(3–14)	(5–15) **	(5–16) ***	
SBI	0,10±0,01	0,70±0,05	1,20±0,09	1,25±0,10	
	(0–0,4)	(0,2–1,2) ***	(0,8–1,9) ***	(0,8–2) ***	

 Table 3. Long-term results of using the Loroben solution in patients with chronic catarrhal gingivitis

*Note:* Statistical significance of the difference with the measurements after treatment: \* - p < 0.05

In children with CCG, included in the main group, after 3 months in 28 patients, the PMA index was  $6.8\pm0.6$ , the SBI index was  $0.70\pm0.05$ . The improvement in the condition of the periodontium to such an extent occurred against the background of the oral hygiene normalization.

After 6–12 months in children with CCG, included in the main group, the clinical state of the oral cavity mainly did not change. In rare cases, negative changes were observed when parental control over hygiene was weakened. Such cases were corrected by improving hygiene and prescribing proper procedures.

In children with MFCP, included in the main group, the clinical and objective results, one might say, were similar. Despite a slight increase in the PMA and SBI indices, after 3–6–12 months they were 3.9 times lower than the initial results.

## DISCUSSION

Chronic diseases of periodontal tissues still occupy major part among diseases of the oral cavity of children. Treatment of these diseases has always been in the field of interest of dentists. So, the main objective of the study was to reveal effectiveness of Loroben solution application in treatment of chronic catarrhal gingivitis and mild form of chronic periodontitis.

So, after continuous application of Loroben solution in patients with CCG on the 3<sup>rd</sup> day of treatment major complaints reduced, while in 25.7% of examined patients slight gingival bleeding was observed. However, on the 14<sup>th</sup> day of treatment none of the patients had any complaint and the clinical examination revealed elimination of all signs of chronic catarrhal gingivitis.

In the group of patients with MFCP the results were almost same. So, on the 3<sup>rd</sup> day of treatment only

of treatment none of the patients had any complaint. During clinical examination neither signs of inflammation, nor bleeding on probing were observed.

Long-term results have also shown positive changes. So, in children with chronic catarrhal gingivitis after 3 months clinical examination revealed no visually detectable signs of inflammation and gingival bleeding. Even after 6–12 months the condition of the oral cavity in patients with chronic catarrhal gingivitis mainly didn't change.

In the group of patients with mild form of chronic periodontitis the results of clinical and objective examinations were almost same with the results of chronic catarrhal gingivitis. Even though PMA and SBI indices were slightly increased at the beginning, after 3–6–12 months these marks were 3.9 times lower than the initial results.

The main limitation for the study was impossibility to control the implementation of the rules of personal oral hygiene in the surveyed group of patients, so the occurrence of the negative outcome in some of the patients CCG and MFCP was the result of the non-compliance to the rules of personal oral hygiene, lack of parental control, interruption of the treatment.

Also, should be mentioned, that immediately after cleaning of the dental plaque and application of Loroben drug to the gingival sulcus area, a significant change in the qualitative and quantitative composition of the oral microflora was observed in patients with CCG and MFCP. More than that, the results of laboratory studies show that usage of Loroben solution for prevention and treatment of periodontal diseases leads to the significant recovery of the functional activity of local immune factors. Based on the results of clinical, immunological studies and objective tests, it can be concluded that the use of Loroben solution is effective in the treatment and prevention of periodontal diseases in children.

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