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EDITORIAL

Dr. Georg Tyminski
Editor-in-Chief

Dear colleagues,

We proudly present the latest issue of Archiv Euromedica. Starting from this year our journal will be published every two months. It became possible thanks to the increased efforts of our authors, reviewers and the editorial team.

For a year and a half the world has been combating the covid-19 pandemic. This required huge resources – financial, human and intellectual. However, the life is going on and researches work on other fundamental problems.

The new issue highlights papers on a wide range of medical topics which were somewhat overshadowed by the pandemic but now they are back.

I would like to draw attention to the paper from Iranian colleagues (Zahedan University of Medical Sciences, Zahedan, Iran) who carried out a cross-sectional study on epidemiological factors associated with the risk of stroke in people under age 50. The topic is very interesting since the study took place during the pandemic and it is known that COVID-19 patients developed acute ischemic stroke. The risk factors are actively studied at the moment. We hope that the authors will continue their research and describe the influence of COVID-19 on frequency of strokes in these age groups.

Nevertheless, older patients are more likely to have multiple comorbidities increasing their risk of ischemic stroke in the setting of COVID-19 infection. However, there are findings suggesting that COVID-19-positive patients presenting with large vessel occlusion (LVO) had lower rates of pre-existing cardiac comorbidities and were actually younger than their COVID-19-negative counterparts. Furthermore, three studies have found COVID-19 infection to be an independent risk factor for both acute ischemic stroke and LVO.

We would like to underline the role our journal plays in uniting and reconciling. Some countries are being in the state of conflict (Russia and Ukraine, Armenia and Azerbaijan) but the authors representing these countries continue to publish their works in the journal. This helps preserve the ties between the scientists and it will help restore them faster.

The new issue comprises the works of the international team of authors who offer original solutions for a number of medical issues. We strongly hope that the findings can be used in clinical practice.

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A SURVEY ON WORKING ENVIRONMENT, PERFORMANCE AND SATISFACTION AMONG PEDIATRIC SONOGRAPHERS IN RUSSIA: FINDINGS AND DISCUSSION

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ABSTRACT — Conducting sociological investigation is an important condition for the implementing effective and targeted health policy. However, such studies, regarding ultrasonic diagnostics specialists are not enough. The article deals with analysis of results of the survey of ultrasonic diagnostic specialists on the issue of evaluation the quality of services provided for ultrasound diagnostics. Among the main factors affecting the quality of ultrasound diagnostics in Russia, doctors' workload and inadequate material and technical base were often noted. Surveys of specialists allow to identify problem areas of an organizational, informational and technological nature in the work of the ultrasound service and take measures to eliminate them.

KEYWORDS — children, ultrasonic diagnostics specialists, quality ultrasonic diagnostics, quality, sociologic survey.

INTRODUCTION

Despite the importance of the problem of quality of medical care, according to majority of researchers, the problem is still unsolved due to its versatility [8, 10]. The problem comprises such categories as qualification, timeliness, availability, and compliance with medical and economic standards for achieving the final result of treatment [4, 5, 7, 9].

During last 60 years' biomedical applications of ultrasound have experienced tremendous growth. At any time, patient safety was an important issue from the beginning, the study of methods for measuring exposure levels, and their relationship to possible biological effects, paralleled the growth of the various therapeutic and diagnostic techniques. The continuously developing conditions of use have presented a range of exposure measurement challenges, and the sensors and techniques used to evaluate ultrasound fields have had to evolve as new or expanded clinical applications have emerged. These conditions demand continuous improvement and expansion of the competence of experts of this sphere of medicine — ultrasonographers and also continuous updating of material support of treatment and prevention facilities for the purpose of delivery of health care according to the existing medico-economic standards updated regularly.

According to the order of Ministry of health of RF from May 10, 2017 No. 203n "On approval of criteria for evaluating the quality of medical care" "quality criteria are applied to assess timeliness of medical care, the correct choice of methods of prevention, diagnosis, treatment and rehabilitation, the achievement of the planned results" [6].

Ultrasound is one of the most common additional methods of examination in pediatric practice, as it is an informative, non-invasive, easily accessible, safe and less expensive diagnostic method than other imaging studies. With high specificity and sensitivity, ultrasound is ranked first as an imaging method in pediatric practice.

When examining children, the ultrasound technique is subject to various requirements with respect to imaging, while in pediatric practice it is necessary to note the increased sensitivity of children to radiation, which limits the possibility of using other techniques. One important aspect of the continuous improvement of medical imaging is the growing potential of ultrasound diagnostics. The use of ultrasound diagnostics as a highly informative, non-invasive, safe method of studying children can help minimize the level of radiation obtained, replacing or limiting other types of imaging. This important task, especially in childhood, can only be achieved by providing specialized qualified pediatric care. Since children are less inclined to cooperate, special treatment of young patients, as well as the environment and conditions in which the child finds himself during the procedure, is necessary to achieve optimal results. Accordingly, the level of equipment of medical institutions providing pediatric care should be the highest, exactly as the qualification of the medical employers of the institution.

Despite the fact that ultrasound in Russian medical institutions is one of the most popular diagnostic methods in various spheres of medicine, the problem of adapting to new socio-economic conditions remains unchanged. In practical terms, sociological investigations are an important condition for an effective and targeted health policy [3]. However, only a few of such studies have been conducted, especially in relation to ultrasound diagnostics doctors.

At the moment, the task of establishing a new system of training of radiation specialists, their subsequent state accreditation and their further development in the framework of the system of continuing postgraduate education still remains; restructuring of the organization and equipping of radiation diagnostics departments at both the outpatient and polyclinic level; creation of appropriate technical conditions for ultrasound research by specialists. Similar problems are faced by medical organizations in Europe. However, in the USA and European countries, more attention is still paid to the issue of training of medical employers - special programs and courses are being developed, which contributes to a rapid and qualitative improvement in the level of qualifications of specialists. Depending on the organization of the health system in different countries, ultrasound diagnostics can be performed by both doctors and general practitioners. According the study of Camilla Aakjær Andersen the availability and use of ultrasound examinations in primary care differs between countries: experts have previously estimated that the proportion of primary care users across 20 countries of Europe varies from less than 1% to 67%,15 and availability of ultrasonography varies from 4% to 58% in the Nordic countries alone [1]. However, the association between larger clinics and access to ultrasonography may also be explained by the multidisciplinary nature of some larger clinics. Some countries, for example, Finland, Spain, Sweden and England, have multidisciplinary teams working in primary care, while others, for example, Switzerland, Romania, Norway, Germany, Denmark and Bulgaria, tend to have less staff [2]. Otherwise, in the Russian Federation despite the huge surface areas there is a unified health system where highly qualified specialists are concentrated in larger municipalities and urban centers.

The purpose of this study was to study the opinion of medical specialists about the quality of ultrasound diagnostics in children.

MATERIALS AND METHODS

The study consisted of a survey of 196 ultrasound diagnostics specialists from medical institutions in Moscow and the Moscow region with the help of specially designed questionnaire consisted of the general characteristics of respondents, assessment of activity of their medical organization, including ultrasonic diagnostics, satisfaction with their work, as well as possible directions for improving the ultrasound service.

The majority of respondents (67.7 %) were women, whose average age was 38.6±10.2 years (men – 36.4±7.7 years, women — 39.9±11.5 years). The majority of respondents (51.3%) worked in hospitals, 28.7% worked in polyclinics, and 20.0% — in private medical centers. The opinion of doctors and their activities were considered depending on the length of work in the specialty.

Mathematical processing of obtained data was carried out with the help of variational statistics. The confidence interval for the average values was calculated with a specific confidence level of 0.95. The student's parametric criterion was used to assess the reliability of differences. The results were processed using the statistical software package Statistica V. 6.1. and the program Microsoft Office Excel 2010.

RESULTS AND DISCUSSION

Doctors of ultrasonic diagnostics were asked to place the criteria, characterizing its quality in ranking order (with an assessment equal to one corresponding to the first rank). As can be seen on Table 1, the most significant criteria, according to the respondents, were effectiveness (2.17±1.7), availability (2.29±1.42) and timeliness (2.98±1.53). Subsequent places were occupied by security (3.27±1.58) and mobility (3.96±1.64). The last place were given by respondents to continuity (5.43±1.46) and economical effectiveness (5.66±1.67).

Table 1. *Rank distribution of criteria that characterize the quality and advantage of ultrasound diagnostics, according to ultrasound diagnostics doctors (M±m)*

Criteria	M±m
Effectiveness	2,17±1,7
Availability	2,29±1,42
Timelessness	2,98±1,53
Security	3,27±1,58
Mobility	3,96±1,64
Continuity	5,43±1,46
Economical effectiveness	5,66±1,67
Others	2,15±1,74

The opinion of doctors about the significance of criteria that characterize the quality and advantage of ultrasound diagnostics changed with the accumulation of experience. The significance of such criteria as performance and timeliness decreased with age. With less than 5 years of professional experience, they were ranked first and second, respectively, and with more than 25 years of experience, they were ranked third and forth. The distribution of other criteria did not change significantly — within the same rank. Similarly, respondents were asked to rank incentives for doctors to improve the quality of ultrasound diagnostics (Table 2) the analysis of questionnaires showed that the most significant incentive for survey participants was material interest (1.37±1.17). The second ranking places were taken by improving the material and technical base (2.33±1.32) and reducing the workload of ultrasound diagnostics doctors (2.67±1.38). Even few respondents were attracted to the possibility of career growth (3.74±1.44) and the atmosphere in the team (4.23±1.42). In the last place was such an incentive as a reduction in working hours (4.76±1.42).

Table 2. *Rank distribution of incentives for improving the quality of ultrasound diagnostics, according to ultrasound diagnostics doctors (M±m)*

Motivations	M±m
The financial interest of doctors of ultrasonic diagnostics	1,37±1,17
Improving of material and technical base	2,33±1,32
Reducing the workload of ultrasound diagnostics doctors	2,67±1,38
The opportunity for career growth	3,74±1,44
Atmosphere in collective	4,23±1,42
The reduction of working time	4,76±1,42
Others	1,86±1,40

As for material interest of doctors to improve the quality of ultrasonic diagnostics, the survey showed that 32.7 % of respondents are not satisfied with the existing system of remuneration. 15.4% of respondents were fully satisfied with their salary and 51.9 % were partially satisfied.

The evaluation of the significance of incentives for doctors to improve the quality of ultrasound diagnostics, depending on the length of service, did not change very significantly. We can say that there has been a tendency to reduce the significance of many incentives, in addition to the possibility of career growth and reduced working hours. In the first case, it did not change, in the second — the interest increased.

As a result of the survey, it was found that the majority (89.9%) of doctors gave a positive assessment of the quality of ultrasound diagnostics in their medical organization (high quality — 19.1%, good quality — 39.9%, satisfactory quality — 30.9%). The percentage of respondents who negatively assessed the quality of ultrasound diagnostics in their medical organization was 10.1%.

The opinion of doctors about the quality of ultrasound diagnostics in their medical organizations differed significantly depending on the length of work in the specialty. For example, with less than 5 years of experience, the majority of respondents (43.3%) considered it satisfactory, and in all other cases — good. At the same time, with experience, the share of doctors giving a good assessment significantly increased: with experience up to 5 years 16.4 %, 5–10 years — 48.3%, 10–15 years — 57.1%, more than 25 years 72.7% ($p<0.05$).

More than one-third (39.4 %) of ultrasound diagnostics doctors who took part in the study reported that their medical organization was evaluating the quality of ultrasound diagnostics, and 32.5% of respondents believed that it was not 28.1% of doctors did not have such information.

In the course of the survey, the question about awareness of medical professionals about the results of their organization's quality assessment of underground diagnostics was clarified. About half (45.5%) doctors answered on this question reported receiving such information, 21.2% did not know anything and 33.3% found it difficult to give an answer about their awareness.

Among the main factors affecting on the quality of ultrasound diagnostics, respondents most often noted the workload of ultrasound diagnostics doctors (in 64.8% of cases) and a weak material and technical base (in 53.1%). Equally often (in 46.4%), such factors as insufficient funding and lack of medical staff were mentioned. It should be noted that not more than one third of the respondents considered the insufficient level of qualification and staffing of ultrasound diagnostics and clinical specialties as factors affecting the quality of ultrasound diagnostics (Table 3).

CONCLUSION

One of the crucial circumstances of using sociological methods is that data from objective medical research, medical statistics, or other summary information obtained from patients ' requests are not able to fully characterize the actual scale and determinants of the problem. Surveys of doctors allow you to identify

Table 3. *The main factors affecting on the quality of ultrasound diagnostics in general, according to ultrasonic diagnostics specialists (in %)*

Factors	%
Insufficient financing	46,4
Weak material and technical base	53,1
Lack of staff for ultrasound diagnostics	46,4
Insufficient level of qualification of ultrasound diagnostics doctors	33,7
The workload of doctors of ultrasonic diagnostics	64,8
Insufficient number of doctors in clinical specialties	27,0
Insufficient level of qualification of doctors of clinical specialties	26,5
Others	9,2

problem areas of organizational, informational, and technological nature in the work of medical organizations and take measures to eliminate them. So, as a result of this study, a number of problems were identified in the course of such highly specialized medical care as ultrasound diagnostics, namely, factors affecting the decrease in the quality of care - the high workload of the doctor and the weak material and technical equipment of medical institutions in the opinion of the specialists themselves. The solution of the identified difficulties by the leadership of medical and prophylactic institutions at the local level, as well as the improvement of the health care system itself as a whole, will contribute to an increase in the availability and quality of medical care, including in the field of ultrasound diagnostics.

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

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THE EPIDEMIOLOGICAL PATTERN OF FACTORS ASSOCIATED WITH ISCHEMIC STROKE IN PATIENTS UNDER 50 YEARS OF AGE: A CROSS-SECTIONAL STUDY

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ABSTRACT — Objective: The aim of the present study was to investigate the epidemiological pattern of factors associated with ischemic stroke among patients under 50 years old. Methods: The present cross-sectional study was performed on 197 Stroke patients. Individuals with confirmed ischemic stroke based on of CT scan were included in the study. Demographic information included age, sex, history of smoking, place of residence, season of disease incidence, history of contraceptive use in women, history of hypertension, having high blood pressure at the onset of diabetes, hyperlipidemia, heart disease and patient weight. Finally, the collected data was analyzed using SPSS ver. 22. Results: The mean age of participants was 40.18 years. The mean weight, height, body mass index (BMI) were 79.83, 168.63, and 28.12, respectively. A total of 60.4% of the participants were male and 39.6% were female. Moreover, 66.9% were urban residents, 38.6% had complications in winter, 67% were smokers, and 50% used contraceptives. The history of hypertension, diabetes, hyperlipidemia, and heart disease was seen in 69%, 49.7, 66%, and 73.6% of cases, respectively. Also, 53.8% of patients had high blood pressure upon admission. Conclusion: The present study showed that a history of heart disease and a history of hypertension in the past and nicotine use are very common in people with stroke. Considering the above mentioned epidemiological factors, it is recommended to prioritize the mentioned factors when identifying new cases.

KEYWORDS — Ischemic stroke; Risk factors; Epidemiology.

INTRODUCTION

Today, stroke is one of the most important diseases in the world. According to the GBD study, stroke accounts for more than 5.5 million deaths and is regarded as the second leading cause of death in the world in 2019. Strokes affected more than 13 million people worldwide in 2019 [1]. World health organization (WHO) defines stroke as rapidly developed clinical signs of focal (or global) disturbance of cerebral function, lasting more than 24 h or leading to death, with no apparent cause other than of vascular origin [2].

Although stroke mortality rates have declined worldwide over the past two decades, the absolute number of people suffers from stroke each year, live with its consequences, or even die. Its prevalence increases due to increasing aging and population growth [3]. It is estimated that 15 million people suffer from stroke each year. Of these, about six million die and another five million become suffer from permanent disability [4]. Life years due to illness, disability or premature death (DALYs) due to stroke are estimated at 44 years per year and are expected to increase to 61 million by 2020 [4]. The global prevalence of stroke is also expected to increase due to an increase in the number of people over 60 years of age of and older. This prevalence is expected to more than double by 2050 and more than triple by 2100, reaching from 901 million in 2015 to 2.1 billion in 2050 and 3.2 billion in 2100 [5].

In low-and middle-income countries (LMICs), the incidence of stroke is rising, and research shows that stroke-related mortality tripled between 2002 and 2020 in Latin America, the Middle East, and sub-Saharan Africa [6, 7]. While the same mortality is increasing in low-income countries compared to high-income countries, and there is a much greater reduction in stroke mortality in high-income countries as compared to low-income countries [8].

Strokes are classified into ischemic and hemorrhagic types based on the underlying pathology [8]. To ensure accurate classification, computed tomography (CT) or magnetic resonance imaging (MRI) as brain imaging techniques are required. Hemorrhagic stroke occurs due to a disorder of the cerebral artery wall and causes intracranial hemorrhage. Hemorrhagic stroke can be classified as intracerebral hemorrhage or subarachnoid hemorrhage based on the site of the hemorrhage. With regard to ischemic stroke, a blood

vessel is blocked, leading to sudden loss of blood flow, local cerebral ischemia, and cell death [9, 10]. The global prevalence of ischemic stroke is twice as high as that of stroke [11]; however, ischemic stroke accounts for about 85% of all strokes in high-income countries [12].

There are several known risk factors for stroke. Age is an important and unchangeable factor. According to a systematic review and meta-analysis, cardiac embolism, atherosclerosis of large arteries (such as carotid artery stenosis), obstruction of small arteries, unknown causes, unusual causes (dissection or vasculitis) account for 22%, 23%, 22%, 26%, and 3% of ischemic strokes, respectively [13].

Various studies show that hypertension, smoking, obesity, poor diet, physical inactivity, diabetes, alcohol consumption, psychological stress and depression, hyperlipidemia cardiac causes (e.g. atrial fibrillation) increase the risk of stroke [13].

Despite many previous individual studies in different regions so far, there have been few studies in this region of Iran. On the other hand, knowing the more accurate course of this disease can help us determine the importance of these factors. The aim of the present study was to determine the epidemiological pattern of risk factors for ischemic stroke among patients under 50 years of age in southeastern Iran.

METHODS

This descriptive study was performed on 197 patients with ischemic stroke referred to an educational center (Imam Ali Hospital) affiliated to Zahedan University of Medical Sciences in southeastern Iran from March 20, 2020 to December 10, 2020. Inclusion criteria included patients aged 12 and 60 years, and having consent to participate in the study. In order to determine the sample size, all patients with confirmed stroke approved by a neurologist were included in the present study.

INSTRUMENT

Data collection was carried out using a researcher-made tool including questions on age, sex, place of residence, season of disease incidence, weight, height, BMI, history of smoking and contraceptive use in women, hypertension, diabetes, hyperlipidemia and history of heart disease.

DATA COLLECTION

Data collection was carried out after obtaining permissions from the Ethics Committee. To this end, the researcher referred to the neurology department for one year and all patients with confirmed ischemic stroke based on CT results admitted to Ali Ibn Abitaleb Hospital of Zahedan were enrolled in the study. Stroke diagnosis and differentiation was carried out by a neurologist based on CT and MRI. Blood pressure higher> 140.90 mm Hg upon admission was considered as hypertension. Diabetes in patients was defined as random blood sugar> 200 mg/dL or FBS> 126 mg/dL or receiving common diabetes medications. Hyperlipidemia was defined as total cholesterol ≥ 221 mg/dL. Patients were given 15 minutes to complete the questionnaires. The questionnaires were collected by interview in the case of illiterate patients.

ETHICAL CONSIDERATIONS

The present study has been approved by the Ethics Committee of Zahedan University of Medical Sciences under the Ethic Code. Written and oral consent was received from all participants. They are assured that their information will remain confidential. The STROBE checklist was used to report the study [14].

DATA ANALYSIS

Data analysis was conducted using SPSS Ver.22. Descriptive statistical tests (mean, standard deviation, frequency and percentage) were used for quantitative variables. Analytical tests (χ^2) were used to describe the demographic characteristics of the participants. Kolmogrove-Smirnov test was also used to evaluate the data distribution. P-value<0.05 was considered statistically significant.

RESULTS

A total of 197 patients entered the final phase. The mean ± SD of subjects' age was 40.18 ± 5.67 years. Mean weight, height, BMI values were 79.83, 168.63, and 28.12, respectively. Most participants were male (60.4%), urban residents (66.9%), had a history of nicotine use (67%), took contraceptive pills (50%), and had a history of hypertension (69%), diabetes (49.7%), hyperlipidemia (66%), and heart disease (73.6%), and were infected in winter (38.1%) (Table 1).

DISCUSSION

In the present study, risk factors such as male sex, nicotine use, history of hypertension, hyperlipidemia, hypertension upon admission, high BMI were all epidemiological factors with a relatively high prevalence in people with stroke. In the study of risk factors for stroke among people aged 15-45 years admitted to Ayatollah Rouhani Hospital in Babol during 2009-2011, Ahmadi et al. reported that mean± SD of patients' age was 38.09 ± 6.11 years, of which 34 (52.3%) were male and the rest were female. The most common stroke was ischemic stroke (n=61 cases, 93.8%), most of which were embolic (n=38 cases, 58.5%) and

Table 1. *Demographic characteristics of the participants*

Variable	Mean ±standard deviation	Number	Percentage
Age	40.18± 5.67	-	-
Weight	79.83 ± 10.87	-	-
Height	168.63 ± 7.88	-	-
BMI	28.12 ± 4.82	-	-
Sex(Male)	-	119	60.4
Sex(Female)	-	78	39.6
Place of residence (city)	-	112	56.9
Place of residence(village)	-	85	43.1
Season (Winter)	-	76	38.6
Season (Autumn)	-	72	36.5
Season (Spring)	-	28	14.2
Season (Summer)	-	21	10.7
Nicotine use (yes)	-	132	67
Nicotine use (no)	-	65	33
Contraceptive use (yes)	-	39	50
Contraceptive use (no)	-	39	50
Previous hypertension (yes)	-	136	69
Previous hypertension (no)	-	61	31
Hypertension upon admission (yes)	-	106	53.8
Hypertension upon admission (no)	-	91	46.2
Diabetes (has)	-	98	49.7
Diabetes (no)	-	99	50.3
Hyperlipidemia (yes)	-	130	66
Hyperlipidemia (no)	-	67	34
History of heart disease (yes)	-	145	73.6
History of heart disease (no)	-	52	26.4

hemorrhagic strokes (6.2%) and transient ischemic attack (TIA) (10.8%). The risk factors included hypertension (n=23 cases, 35.4%), heart diseases (n=20 cases, 30.8%), including ischemic heart disease (n=7 patients,10.8%) and patent foramen ovale (n=6 cases) and mitral valve stenosis (n=6 cases). and the rest included other cardiac causes. The prevalence of substance and alcohol abuse, hyperlipidemia, diabetes, blood disorders, increased coagulation was 18 cases (28.1%), 15 cases (23.1%), 13 cases (20%), 12 cases (18.5%), with 6 cases (9.2%), respectively. Other causes were seen in 13.8% of cases and there was no specific risk factor in 7.7% of cases [15].

According to the results of the present study, ischemic stroke is the most common type of stroke and hypertension, heart disorders, substance abuse, hyperlipidemia and diabetes are the most risk factors for stroke. O'Donnell et al. (2016) found that these ten potentially modifiable risk factors accounted for about 90% of all strokes. Hypertension is more associated with intracerebral hemorrhage than ischemic stroke, while smoking, diabetes, and apolipoprotein-related causes, and cardiac causes are more commonly associated with ischemic stroke. However, hypertension is the most important modifiable risk factor for both hemorrhagic and ischemic strokes [16]. In a study of the risk factor and etiology of ischemic stroke among young adults, Renna et al. (2014) reported that the mean ± SD of patients' age was 41± 8 years [17]. The most common risk factors included dyslipidemia (52.7%), smoking (47.3%), hypertension (39.3%) and patent foramen ovale (PFO) (32.8%), large artery atherosclerosis leading to stroke (n=17 patients, 11.3%). Cardioembolism occurred in 36 patients (24%), most of whom showed PFO on transesophageal echocardiography (TEE). Small vessel occlusion was diagnosed in 12 patients (8%) and all of them had hypertension and most of other risk factors. A total of 41 patients (27.3%) showed a stroke of other known causes and 44 patients (29.3%) showed a stroke of unknown causes. The three-year survival rate was 96.8% and recurrent stroke occurred in only three cases [17] The present study is consistent with these studies. Considering that studies have shown that hypertension is an important risk factor for patients due to the subsequent pressure on the heart and other blood vessels [18,19]. Nicotine consumption is also another risk factor for hypertension due to the creating inflammatory conditions and increasing inflammatory cytokines in the body. There have been many studies on nicotine consumption and results showed a evident relationship between it and the risk of stroke [20]. Diabetes mellitus is one of the most important conditions leading to stroke with regard to its involvement in the development of microvascular and macrovascular disorders. It should be considered that the disease duration and the degree of control over the disease are important factors [2]. The present study also showed that people with a history of stroke had BMI values higher than normal range. As studies have shown, the presence of BMI is a risk factor for vascular disease, which is even directly related to the prognosis of recovery. Considering that BMI itself is an index to show the fat distribution and also indirectly the amount of activity in each individual [21]. The present study showed that a high percentage of hypertensive people upon admission, which causes a reversal cycle to overcome the crisis due to the vascular obstruction and the body's resistance to compensate for this obstruction. The most important limitations of the present study

included low sample size, the short follow-up duration, and the absence of a control group. Therefore, it is recommended to carry out further relevant studies with a larger sample size over a longer period of time.

CONCLUSION

The present study showed that factors such as history of heart disease, history of hypertension, nicotine use, known hyperlipidemia, male sex and urbanization were important risk factors leading to a high prevalence stroke. Therefore, we better recognize the increasing epidemiological significance of these factors.

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CORRECTION OF THROMBOCYTOPOIESIS IN RATS WITH WALKER-256 CARCINOMA USING AN ANTIOXIDANT SUPPLEMENT IN THE SETTING OF CYTOSTATICS

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ABSTRACT — In this study, we've evaluated the myelotoxic effect of treating Walker-256 carcinoma with cytostatics, followed by correction of thrombocytopenia with liposomal and liposome-free mexidol. The study included 60 rats, which, depending on the type of treatment, were divided into 5 groups. We recorded the greatest increase in the number of platelets on the background of liposomal mexidol both on the 3rd and 7th day after the start of chemotherapy. At the end of the monitoring, the myeloprotective effect was 30% higher in the liposomal mexidol compared to its non-liposomal form. Conclusion: Activation of thrombocytopoiesis on the background of cytostatic therapy helps to reduce the complications related to the use of chemotherapy.

KEYWORDS — Walker-256 carcinoma, platelets, megakaryocytes, thrombocytopenia, doxorubicin, mexidol.

INTRODUCTION

Breast cancer occupies the leading place among malignant neoplasms in women [1, 2, 3, 4]. At the same time, cytostatics are widely used in the treatment of breast cancer [5]. In the setting of cytostatic therapy, side effects often develop: anemia, thrombocytopenia, neutropenia, bleeding, etc. [6]. Myelosuppression is the main reason for limiting the dose of cytostatics in the treatment of breast cancer.

It's also known that antitumor agents cause a strong oxidative stress in the body in combination with the depletion of antioxidant protection, which increases the inhibition of the myelopoietic germ [7]. Therefore, the search for effective methods for regulation of thrombocytopenia, which is a common complication in cancer patients receiving cytostatic therapy, poses one of the urgent challenges to modern medicine.

Aim: to estimate the effectiveness of antioxidant therapy in the correction of thrombocytopoiesis in rats with Walker-256 carcinoma on the background of cytostatic therapy.

METHODS

The experimental work was performed on 60 female Wistar rats weighting 160–270 g. All procedures with animals were performed according to the guidelines for the care and use of laboratory animals. The tumor process was modeled by introducing a suspension of Walker-256 (W-256) carcinoma cells under the skin of the rat tail, followed by histological verification of the neoplasm. For cytostatic therapy, we used doxorubicin hydrochloride ("Pharmachemie", the Netherlands) — 0.04% solution, which was diluted with isotonic sodium chloride solution. To correct thrombocytopoiesis in rats with Walker-256 carcinoma, we used Mexidol (Emoxypine); Pharmasoft Pharmaceuticals, Russia) both in its free and liposomal form. In both cases, we used Mexidol at a dose of 50 mg/kg intravenously daily from the beginning of the use of cytostatics for 7 days.

Depending on the chosen treatment, all animals were divided into 4 groups:

group 1 (n=12): intact rats — these animals were not manipulated;

group 2 (n=12): rats with transplanted Walker-256 carcinoma that do not receive medication;

group 3 (n=12): rats with Walker-256 carcinoma receiving doxorubicin hydrochloride 4 mg/kg once intravenously on the 11th day after dissemination of tumor cells;

group 4 (n=12): rats with Walker-256 carcinoma, receiving doxorubicin hydrochloride 4 mg/kg once intravenously on the 11th day after dissemination of tumor cells and liposome-free mexidol at a dose of 50 mg/kg intravenously daily from the beginning of the use of cytostatics;

group 5 (n=12): rats with Walker-256 carcinoma, receiving doxorubicin hydrochloride 4 mg/kg once

intravenously on the 11th day after dissemination of tumor cells and liposomal mexidol at a dose of 50 mg/kg intravenously daily from the beginning of the use of cytostatics.

Liposomes were obtained by phase reversal from lecithin and cholesterol. The drug was encapsulated by the method of passive loading. To create liposomes, a Heidolph rotary evaporator (Germany) and a LISEC extruder (Canada) were used.

On the 3rd and 7th day after the administration of cytostatics, 6 animals from each group were removed from the experiment under general anesthesia with sodium thiopental (50 mg/kg). We counted the number of platelets in the blood of experimental animals in the Goryaev chamber. Using light microscopy, we examined bone marrow smears from the rat femur and counted the number of megakaryocytes.

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 at $p<0.05$.

RESULTS

Administration of doxorubicin to rats with Walker-256 carcinoma led to the development of thrombocytopenia. The dynamics of changes in the number of platelets in the observed groups of rats is shown in Fig. 1. Thus, on Day 3, the most severe thrombocytopenia was observed in group 3: the level of platelets decreased by 37% ($p<0.01$) in relation to intact rats. Besides, in this group of animals, the inhibition of the myeloid growth was expressed almost twice as much by the 7th day of the experiment. In rats that received Mexidol in a liposomal form during a week, a dynamic increase in the number of platelets was observed: by 40.3% in relation to the 3rd group of animals; by 17.7% in relation to the 4th group of animals (Fig.1).

The dynamics of changes in the number of megakaryocytes in the rat bone marrow in all groups is shown on the Fig. 2.

In the bone marrow on the 3rd day after chemotherapy, only in animals of the 3rd group, the number of megakaryocytes significantly decreased by 80% ($p<0.05$). By day 7, the number of megakaryocytes in group 3 exceeded the baseline level in intact rats by 3 times ($p<0.05$, Fig. 2). In the bone marrow on the 3rd day after the administration of cytostatics in the 4th group of rats, the content of megakaryocytes did not significantly differ from the indicators for this criterion among intact rats. On the 7th day, the number of megakaryocytes in the 5th group of animals was significantly higher by 58.3% compared to the initial values; by 56.9% in relation to the second group of animals; by 36.3% in relation to the 4th group ($p<0.05$, Fig. 2).

DISCUSSION

The main toxic effects of doxorubicin are anemia, leukopenia, and thrombocytopenia. Moreover, the hematological toxicity of this drug sometimes reaches the 4th degree [8]. Our study showed that the exposure to doxorubicin in rats with Walker-256 carcinoma led to the development of thrombocytopenia with a tendency to reduce the number of megakaryocytes in the bone marrow by the 3rd day after the initiation of doxorubicin therapy. However, thrombocytopenia can be caused not only by the inhibition of platelet growth of hematopoiesis, but also by the direct damaging effect of cytostatics on platelets. It is known that reactive oxygen species cause platelet apoptosis via the Extracellular Receptor Kinase signaling pathway (ERK), which is characteristic of some cytostatics [7]. Such a phenomenon as: an increase in the number of megakaryocytes in the bone marrow in groups 3, 4 and 5 of rats by the 7th day after the after the initiation of doxorubicin therapy, we take as a compensatory

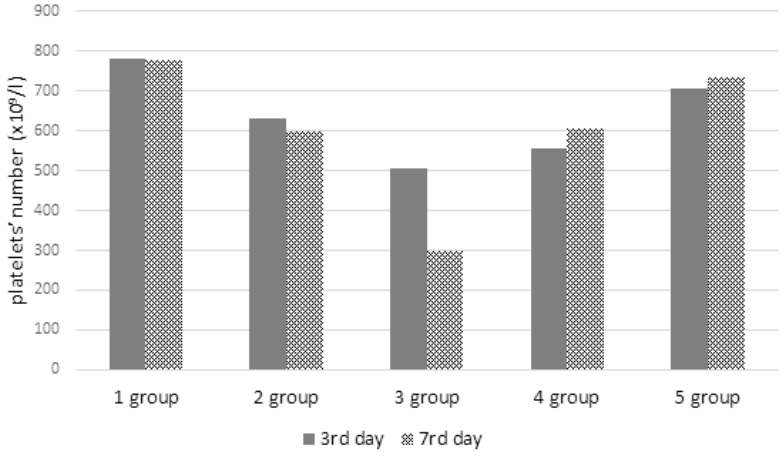

Fig. 1. Dynamics of changes in the number of platelets in the observed groups of rats

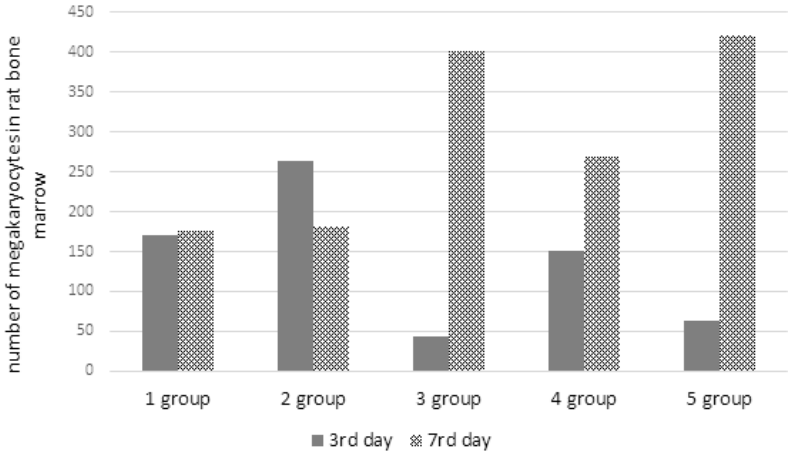

Fig. 2. *Changes in megakaryocyte volume in the rat bone marrow in all groups*

reaction in response to the developed and persistent thrombocytopenia. However, the liposomal mexidol had a greater myeloprotective effect compared to its free form with advantage of 30%, which confirms higher effectiveness of the drug.

Thus, our study showed that the preservation of the initial number of platelets in the peripheral blood on the 7th day after the cytostatic treatment supplemented with the use of liposomal mexidol resulted in a significant increase in the number of megakaryocytes in the rat bone marrow compared to the control group, which may indicate the activation of thrombocytopoiesis.

CONCLUSION

Liposomal mexidol (50 mg/kg), in contrast to its free form, prevents the development of thrombocytopenia induced by cytostatic therapy in rats with Walker-256 carcinoma. Activation of thrombocytopoiesis during cytostatic therapy helps to reduce the complications, related to the use of chemotherapy.

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MORPHOMETRIC STUDY OF ISOLATED DOGIEL TYPE II CELLS OF THE INTERMUSCULAR PLEXUS

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ABSTRACT — Aim of the study was to calculate the morphometric parameters of isolated Dogiel type II cells from the intermuscular plexus of the small intestine.
Materials and methods: 3D models of the oval-shaped Dogiel type II cells from the intermuscular plexus of a rat (n = 1) were constructed and studied. Neurocytes were identified by the impregnation method on the frontal and horizontal sections of the wall of the small intestine.
Results: The results showed that the total number of the nodes in the virtual model was 36534, and the mesh elements — 156595. The resulting 3D model of the cell and nucleus was reduced 900 times to obtain a three-dimensional cell and nucleus with absolute dimensions, with a ratio of 1:1 to their true size. The volume of Dogiel type II cell was 2785.11 μm^3, the volume of the nucleus was 647.7 μm^3 and the volume of its perikaryon was 2785.11 μm^3.
Conclusion: Dogiel type II cells from the intermuscular plexus of the rat small intestine has an ovoid shaped three-dimensional structure. These cells are flattened in transverse direction and elongated in longitudinal direction.

KEYWORDS — Dogiel type II cells, intermuscular plexus, small intestine, volume of perikaryon, volume of nucleus.

INTRODUCTION

The vegetative ganglia are a collection of the numerous multipolar neurons. The size of the vegetative ganglia varies significantly. There are large, medium-sized, small and very small (microganglia) ganglia [1].

Dogiel cells, neurons of the autonomic ganglia. There are three types of cells (I–III). Type I cells (efferent neuron with a long axon) are neurons with short dendrites (located within the ganglion). Type II cells (multipolar, afferent neurons) are a neuron with a long neurite and dendrites; the latter go beyond the ganglion, functionally they are the sensitive (afferent) neurons. Type III cells (associative, intercalary neurons) are the neurons with a long neurite (can reach other autonomic ganglia), dendrites are short (within one ganglion) [2].

Dogiel type II cells, as suggested by A.S. Dogiel, actually perform a sensory function [2]. Their activity is due to the direct action of serotonin and, possibly, the effect of acetylcholine [3]. It is also possible that there are special functional connections between the neurocytes of the intermuscular and submucosal plexuses [4]. However, the data on the morphometric parameters of Dogiel type II cells of both the intermuscular and submucosal plexuses are extremely contradictory, due to the large number (about 40) of the most typical shape of their perikaryon [5]. Moreover, it is believed that neurocytes in metasympathetic ganglia are flat in structure and their thickness is only a few microns [6].

The aim of this study was to calculate the morphological parameters (volumes of perikaryon and nucleus) of an isolated cell of rat's small intestine.

MATERIAL AND METHODS

The neurocytes of the ganglia of the intermuscular plexus of the small intestine of a mature outbred rat (n = 1) were identified by the universal method of impregnation [7]. After fixing a fragment of the intestinal wall in 10% amethanol formalin (5 days), its frontal and horizontal sections with a thickness of 30.0–40.0 μm were made. Slices with an area of 3.0×5.0 cm were enclosed in Canadian balsam and examined under a 1000DM loess microscope with a digital video system. For morphological analysis, two Dogiel type II cells were selected, visualized on the frontal and horizontal sections of the intestinal wall. The morphometric studies of these cells were carried out in the Ymadei software package, the construction of their 3D models — in the AN5Gs Apace claim v 19.2 software.

RESULTS

The color photographs were taken to assess the volume of the perikaryon and nucleus of isolated Dogiel type II cells located in the frontal and horizontal planes (Fig. 1).

Then the photographs were converted into the BMP raster image format (3DtooLR software environment). The sections of neurocyte bodies located in frontal and horizontal sections were separately cut from these images (Fig. 2).

The resulting images were first combined in a Cartesian coordinate system, and then, after aligning

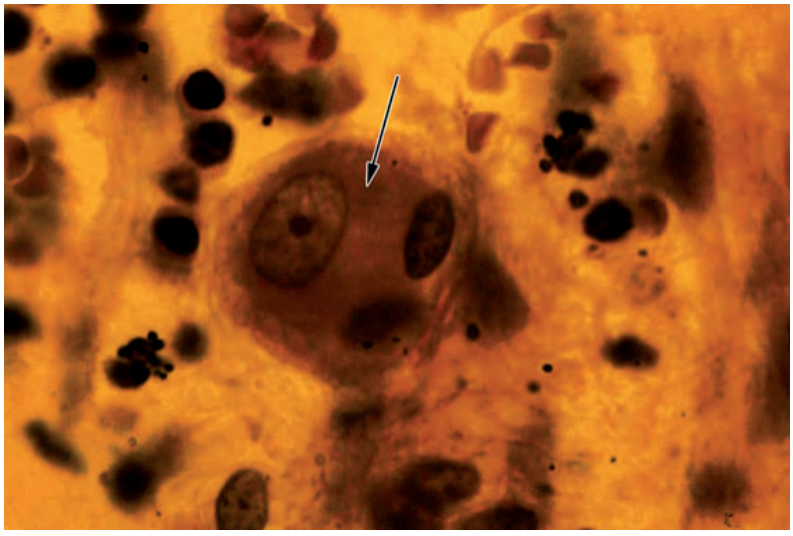

Fig. 1. *Dogiel type II cell in the horizontal plane. A universal method of impregnation. Sw.× 900*

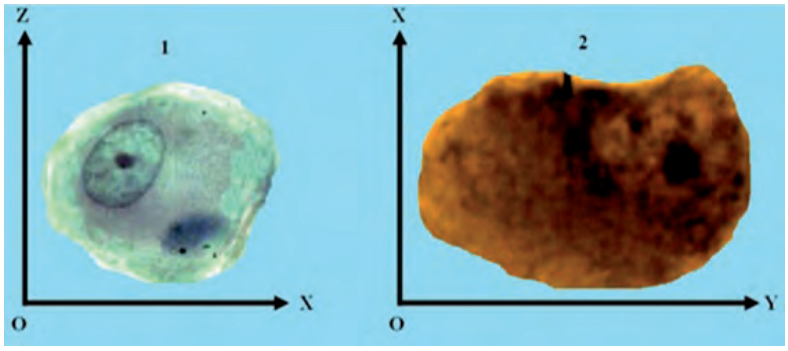

Fig. 2. *Morphometric characteristics of Dogiel type II cells in the Cartesian coordinate system 1) section in the xOz plane; 2) a section in the xOy plane*

the planes and transforming them into a Stl file, into a solid model (in the AutodeskR software environment). The total number of the nodes in the virtual model of Dogiel type II cell was 36534, and the mesh elements were 156595. The resulting 3D model of the cell and nucleus was reduced 900 times in order to obtain a three-dimensional cell and nucleus with absolute dimensions, with a ratio of 1:1 to their true dimensions (Fig. 3).

In the calculations, a number of assumptions were made: 1) the section of the cell in horizontal plane was approximated to a regular shape; 2) the distance between the sections is 0.1% of the cell volume; 3) the material of the investigated volume is isotropic.

The object of the research, the body of the cell H, the overall characteristics of which had to be calculated, is located between two arbitrary planes.

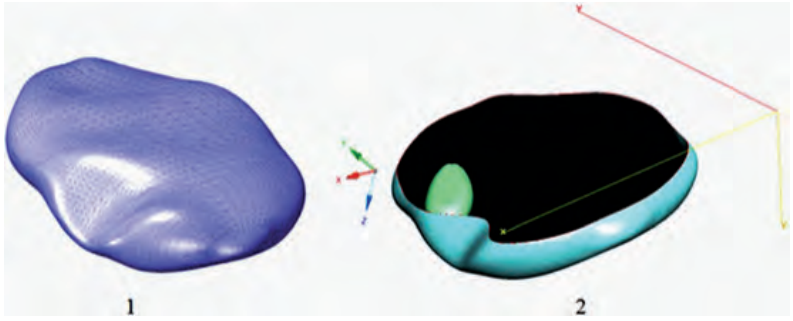

Fig. 3. *Solid-state model of Dogiel type II cell 1) actinometric image; 2) horizontal section of the cell*

The coordinate system is built so that the Ox axis is perpendicular to a and b planes. Letters a and b denote the abscissas of the points of intersection of Ox axis with these planes (a < b). We assume that the body of the cell H is such that its section ϕ (x) is a plane passing through a point with an abscissa (x) and perpendicular to the axis (Ox). Thus, they are an ellipse.

The method used in calculating the volume of the cell consisted in separation of its cross-section into separate areas and in calculating the area of each of them with the subsequent addition of the results.

The following results were obtained: the volume of Dogiel type II cell was 2785.11 μm^3, the volume of the nucleus was 647.7 μm^3, the volume of its perikaryon was 2785.11 μm^3, and the volume of the nucleus was 647.7 μm^3.

DISCUSSION

When studying physiological and pathological processes in biological systems, it will be essential to employ cybernetic principles [8]. It must be admitted that *the pathological process is too complicated to solve its nature without the help of mathematics*. In this regard, at present, the most promising method for the study of histological material is CAE technologies. Moreover, the reconstruction of a neurocyte, due to the complexity of its morphology, requires from 2000 to 3000 measurements of its various parameters [9]. At the same time, it is recognized that the morphometric parameters of the intestinal neurocytes of humans and dogs are practically the same. In the literature, studies of the morphometric parameters of various cells, including neurocytes, using *manual* counting are presented [10]. For this, in some cases, the large and small diameters of the perikaryon and nucleus were measured, and their volumes were calculated using the ellipsond formula (V = Pa2v) [11]. In other cases, the volume of the perikaryon and the nucleus was found by the formula: V = av2; where a — large diameter, b — small diameter. In third cases, the cell volume was determined by the formula V = a2b, in spherical nuclei — V = D30.52, ellipsoidal nuclei — V = (a + b) 2 0.07 [12]. In all cases, there is a few number of the measurements with a "manual" method of counting and their relative low accuracy [10].

FINDINGS

1. The Dogiel type II cell from the intermuscular plexus of the rat's small intestine has an ovoid shaped three-dimensional structure.

2. The cell is flattened in transverse direction and elongated in longitudinal direction.

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EXPERIMENTAL STUDY OF THE PHENOMENON OF MICROORGANISM-ASSOCIATED CRYSTALLOGENESIS

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ABSTRACT — The aim of the study was to clarify the character of the initiatory activity of pathogenic and non-pathogenic microorganisms in regard to salt solutions. We selected colonies of two microorganisms: Escherichia coli (E. coli) and Staphylococcus aureus (St. aureus) [10^6, 10^8, 10^{10}, 10^{12}, 10^{14}, 10^{16}, 10^{18} and 10^{20} CFU/ml of solution]. A 10% solution of sodium chloride was used as the initiated substance, which made it possible to perform a tezigraphic test. On the basis of model experiments with colonies of some pathogenic and non-pathogenic microorganisms, a new phenomenon was confirmed — microbial initiation of crystal formation (the phenomenon of microorganism-associated crystallogenesis). This phenomenon is universal for microagents, but it also has specific features imposed by the species of the microorganism and its current functional state.

KEYWORDS — biocrystallomics, microorganism-associated crystallogenesis, Staphylococcus, Escherichia.

INTRODUCTION

The metabolic activity of various microorganisms is widely discussed in the literature. At the same time, various properties of bacteria are studied and applied in some industries [1, 2, 6, 7]. In these works, numerous facts of the connection of the processes of crystal formation with the activity of microbes are presented. The most studied are representatives of the genus Pseudumonas, which are able to initiate the crystallogenesis of various organic and mineral compounds from solutions [1, 2, 5]. Similar properties were found in other microorganisms (Listeria innocua, Escherichia coli, Helicobacter pylori, Desulfovibrio desulfuricans, Agrobacterium tumefaciens [1–3, 5] etc.). A significant practical interest is the ability of microorganisms to accumulate many metals, including noble metals (gold, silver, platinum) in the form of microcrystals in the perimembrane space or intracellularly [1, 2, 6, 7].

Thus, ***the aim of the study*** was to clarify the character of the initiatory activity of pathogenic and non-pathogenic microorganisms in regard to salt solutions.

MATERIAL AND METHODS

We selected colonies of two microorganisms: Escherichia coli (E. coli) and Staphylococcus aureus (St. aureus). Tthe strains were obtained at the Kirov Research Institute of Hematology and Blood Transfusion. In order to clarify the role of the concentration of the bacterium, the same test amounts of bacteria were used for both microorganisms: 10^6, 10^8, 10^{10}, 10^{12}, 10^{14}, 10^{16}, 10^{18} and 10^{20} CFU/ml of solution. A 10% solution of sodium chloride was used as an initiated substance, which made it possible to perform a tezigraphic test [4]. This allowed to quantify the *initiatory potential* of the bacteria — the ability to influence the crystallization pattern of the salt solution. Statistical processing of the results was performed using variation statistics algorithms, Microsoft Excel 2007 and Statistica 6.1 for Windows.

RESULTS

The study of crystallogenic properties of 10% sodium chloride solution has allowed to establish that there is a dynamics accelerating the expressiveness of shifts in crystal formation of hypertonic saline with an increase in the content of microagent. It is important that this trend applies to both micro-organisms. A more detailed study of the distribution of tezigrams along the line of the increasing initiating dose of the microagent, taking into account all controlled concentrations, revealed a two-stage nature of the reaction of sodium chloride on the metabolic activity of the microorganism. It includes the initial absence of significant variations compared to the reference sample of the reference substance. As the number of microbial bodies increases, a concentration for each microorganism is clearly recorded, which initiates the dynamics of crystallogenesis transformation in the marginal zone. It is expressed in the formation of islands of single crystals surrounding relatively large pyramidal structures formed by sodium chloride. It is essential that the

concentration, at which such changes are detected in Escherichia and Staphylococcus, varies, and it is significantly less in the pathogenic microagent. Thus, for E. coli, this threshold concentration is 10^{12} CFU/ml, while for St. Aureus, even with the minimum (of the studied) number of bacteria (10^6 CFU/ml), significant transformations of the structure of the marginal zone are registered.

The third of the variants of initiation of sodium chloride crystallization by bacteria associated with the number of micro-agents identified by us is the increasing neocrystallogenesis in the central and intermediate zones of the dried samples. Based on this, it is possible to distinguish the second threshold concentration, and its values species-specific (10^{14} CFU/ml for E. coli and 10^{10} CFU/ml for S. aureus).

In accordance with the chosen method of describing dried samples, we performed not only a qualitative, but also a quantitative assessment by applying a system of criteria. The analysis showed that the qualitative transformations associated with the metabolic activity of bacteria are fully confirmed when evaluating the quantitative criteria of the diagnostic test. In particular, the monotonous increase in the level of the main tezigraphic coefficient is the result of first marginal, and then total (extending to all zones of the facia) neocrystallogenesis, reflecting the *initiator potential* of the microorganism. At the same time, the increase in the number of crystal structures is not accompanied by an increase in structure index. Thus, in terms of the main tezigraphic coefficient, and, consequently, in terms of the initiatory capacity (when using a 10% sodium chloride solution as a base substance), St. aureus significantly surpasses E. coli at all the studied concentrations ($p<0.05$), and if the *initiatory potential* of E. coli is insignificant at small values, then it is initially high in staphylococci (increased crystal formation of sodium chloride by 3.5 times). At high concentrations, the clear differentiation between bacteria is somewhat smoothed out due to a pronounced increase in the initiator potential of E. coli.

Similar trends were found when assessing the *correctness* of the crystallogenesis of the sodium chloride solution modulated by microorganisms (according to facia destruction degree), but no significant differences were at low concentrations of bacterial agents ($p<0.05$). In addition, a pathogenic bacterium (in our case, Staphylococcus aureus) at medium and high concentrations has a fairly clear destructive effect on the crystallization of sodium chloride solutions than an Escherichia coli. These changes are verified by correlation analysis ($r=-0.103\pm0.062$).

CONCLUSION

On the basis of model experiments with colonies of some pathogenic and non-pathogenic microorganisms, a new phenomenon was confirmed — microbial initiation of crystal formation (the phenomenon of microorganism-associated crystallogenesis). This phenomenon is universal for microagents, but it also has specific features imposed by the species of the microorganism and its current functional state.

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EFFECT OF CU(II)$_2$(3,5-DIPS)$_4$(H$_2$O)$_3$ ON SURVIVAL, AVERAGE LIFE AND HEMATOLOGICAL INDICATORS IN ANIMALS WITH BURNS III DEGREE

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ABSTRACT — Taking into account the properties of copper: normalization of the endocrine system, participation in the process of hematopoiesis, participation in the synthesis of collagen and elastin, neutralization of toxins, reduction of inflammatory reactions — we used a number of copper chelates to treat burn injuries under experimental conditions.

Before discovering possible healing properties for burns, we quantified the toxicological properties of the substance. For this, the average lethal dose LD50/7LD50/7 which is the dose of a substance at which 50% of animals die within 7 days (after subcutaneous injection of the substance), was calculated by the integration method of H. Behrens in experiments on rats.

In the second series, we caused grade III burn lesions on the backs of epilated animals, 30% of the body surface. The organometallic complex Cu(II)$_2$(3,5-DIPS)$_4$(H$_2$O)$_3$ was injected into the animals intraperitoneally 30 min after the burn injury (for the first time at a dose of 50 mg/kg). From day 2, chelate was administered at a dose of 20 mg/kg. The injection was done once every 2 days for 10 days until the scab falls off.

According to the experimental results, the Cu(II)$_2$(3,5-DIPS)$_4$(H$_2$O)$_3$ metal complex is a compound with low toxicity. When administered subcutaneously in the dose range from 100 mg/kg to 300 mg/kg, behavioral disturbances and death of animals were not observed within 24 hours. We studied 5 hematological indicators: blood clotting time (BCT), the number of leukocytes, erythrocytes, the level of hemoglobin and platelets. Survival and average life expectancy in the group (*burn*) were, respectively, 40% and 18.8 days, and in the group *burn + metal complex* — 60% and 19.2 days, which indicates a positive effect of this metal complex on the body. There was a significant difference in clotting time, leukocyte count and platelet count compared to the control group ($p<0.05$), which indicates the healing properties of the Cu complex in case of burns.

KEYWORDS — thermal burns, blood clotting time, white blood cells, platelets, hemoglobin, red blood cells, metal complexes.

INTRODUCTION

The copper biological role is diverse. Copper is the key component of the enzyme cytochrome oxidase, carrying out cellular respiration in all organs and tissues and is a constituent of vitamins, hormones and pigment substances. Copper has an impact on the synthesis of sex hormones, normalizes the work of the endocrine system, activates insulin. The biogenous role of copper is participating in the processes of hematopoiesis. The trace element taking part in the synthesis of hemoglobin, carrying out the transfer of oxygen in the body, increases the speed of blood circulation. Copper takes part in the synthesis of collagen and elastin, supports skin turgor; without it connective tissue loses its resilience, bones and cartilage lose elasticity. Copper is also important for nerve tissue, it is a constituent part of the myelin sheaths of nerve cells isolating nerve fibers. Copper has an active participation in the metabolism of carbohydrates: activates the oxidation of glucose, slows down the destruction of glycogen in the liver. Copper is of great importance for the immune system. The metal neutralizes the toxins of microorganisms, prolongs the impact of antibacterial drugs, reduces inflammatory reactions.

The use of copper sulphate determines a faster closure of the dermal wounds so the application of copper sulfate has been proposed in regenerative medicine. According to literary sources [7-9,11] and also our early studies [2-6,10,12], several copper-based complexes are of low toxicity and expressed radioprotective properties. In order to identify a possible positive effect on burns, we studied a number of compounds when used internally.

MATERIALS AND METHODS

All experiments were carried out on sexually mature, outbred white rats (a total of 60 animals: 10 rats for each series) weighing an average of 170–190 g.

Before discovering the healing properties in case of burns, we had to carry out quantitative assessments of the toxicological properties of the substance. Determination of the toxicological properties of Cu(II)$_2$(3,5-DIPS)$_4$(H$_2$O)$_3$ is performed to quantitatively evaluate the dose-effect relationship. The

toxicological properties of the Cu complexes are characterized by the calculation of the LD50/7 parameter. LD50/7 is the dose of a substance in case of which the 50% of the animals die within 7 days (after subcutaneous injection of the substance).

For this purpose, the substance was given to the animals in gradually increasing doses, from the maximally ineffective to LD100/7 (i.e. the minimum dose that is absolutely lethal to 100% of the animals within 7 days). The mean lethal dose of LD50/7 was calculated using the integration method of H. Berens in experiments on rats [1].

In the second series, we caused grade III burn lesions on the backs of epilated animals, 30% of the body surface. The organometallic complex $Cu(II)_2(3,5\text{-}DIPS)_4(H_2O)_3$ was injected into the animals intraperitoneally 30 min after the burn injury (for the first time at a dose of 50 mg/kg). From the second day, chelate was administered at a dose of 20 mg/kg. The injection was done once every 2 days for 10 days until the scab falls off.

The data obtained from animals subjected to a burn followed by injection of a chelate were compared with those obtained in the control group (animals exposed only to burn lesions).

Blood samples were taken from the tail vein of animals on days 3, 7, 14 and 30 for hematological examination. Also, visual monitoring of the condition of burn wounds in 2 groups of experimental rats (*burn* and *burn + metal complex*) was carried out. The examinations continued for 3 months until the wounds were completely healed (up to hair growth).

RESULTS

According to experimental results, $Cu(II)_2(3,5\text{-}DIPS)_4(H_2O)_3$ metal complex is a compound with low toxicological properties. In case of subcutaneous injection (100–300 mg/kg), no behavioral disorder or animal death was observed within 24 hours. Only in case of injection of extra high doses (1675 mg/kg and more) did the animals show weakness and inhibited motor functions, often immediately after the injection, which was leading to animal death within a few hours.

Hematological examinations (at a dose of 270 mg/kg) were performed in parallel (5 indicators were studied: blood clotting time (BCT), white blood cells, red blood cells, hemoglobin and platelet levels), the results of which are presented in Table 1, aaccording to which, on the 7th day of the experiment, a significant difference in almost all indicators was observed between the animals that received a large dose of the $Cu(II)_2(3,5\text{-}DIPS)_4(H_2O)_3$ substance, versus the control group (normal, intact animals).

After carrying out a quantitative assessment of the toxicological properties of the substance, we studied the following 2 groups of animals: those subjected to burn injuries followed by injection of a chelate were compared with the data obtained from the control group (animals exposed only to burn injuries).

Made the determination of the survival rate and average life expectancy in these groups. The dynamics of the survival of animals from the groups *pure burn* (1) and *burn + metal complex* (2) was described by regression curves and equations, respectively: $y_1 = 83.67–26.48\lg(x)$, $y_2 = 84.07–21.15\lg(x)$, where y is the percentage of surviving animals, and x is the day of the experiment (and in absolute terms, the survival rate and average life expectancy in subgroup (1) was 40% and 18.8 days, respectively, and in subgroup (2) 60% and 19.2 days), which speaks of the positive effect of this metal complex on the body. This was also confirmed by the dynamics of changes in blood parameters during the entire period of the experiments.

According to the data obtained, the $Cu(II)_2(3,5\text{-}DIPS)_4(H_2O)_3$ complex exhibited effective properties, since throughout the entire period and until the end of the experiment, blood counts in animals with a clean burn and in rats that received burns lesions followed by complex injections were significantly different. There was a significant difference in clotting time, leukocyte count and platelet count compared to the control group ($p < 0.05$), which indicates the healing properties of the Cu- complex in case of burns.

Thus, leukocytosis, usually found after a burn, is not only facilitated by this compound in the early stages of experimental work ($14.1\pm1.05 \cdot 10^9$/l — only with burn lesions, $8.8\pm0.7 \cdot 10^9$/l — with burn lesions with subsequent injections of the drug), but also after 30 days ($12.53\pm0.23 \cdot 10^9$/l — only for burn injuries, $5.93\pm0.6 \cdot 10^9$/l — for burn injuries with subsequent injections of the compound).

The results of other hematological evaluations (groups *burn* (indicated in parentheses) and *burn + chelate*) are presented in Table 2.

On the 80th day after the complete healing of the wound, a visual examination showed that the rejection of the scab was noted at approximately the same time within 12–16 days after the application of the burn. As for wound healing, epithelialization and hair growth, these processes are more intense in the *burn + metal complex* group than in the burn group, which also speaks of the positive effect of this metal complex on the burned body.

CONCLUSION

Based on the results of assessing the survival rate, life expectancy and hematological param-

Table 1. *Hematological indicators during the evaluation of toxicity*

indicator / day	Norm	7	14	30
Blood clotting time (sec)	311.0±19.00	501.0±30.39 (*)	476.0±32.34 (*)	322.0±21.00
White Blood Cells (N/μL)	11500.0±420.0	15400.0±529.15 (*)	14360±591.27 (*)	13500.0±520.0
Platelets (N/μL)	520000±18230	505000±18708.3	534000±22271.06	526000±19430
Hemoglobin (g/L)	138.1±5.82	139.0±1.18	146.0±1.38	139.1±2.82
Red Blood Cells (N/μL)	5823000±278800	5254000±113075.2 (*)	6538000±126427.8 (*)	5852000±217800

Table 2. *Changes in hematological parameters in "burn" (indicated in parentheses) vs "burn+chelate" effect*

day / indicators	3	7	14	21	30
Blood clotting time (sec)	265.0±17.27 (415,0±7,63) (*)	321.25±21.85 (327,5±32,5)	279.38±15.39 (360,0±34,64) (*)	238.33±14.81 (338.5±43.55) (*)	276.67±23.33 (316,7±52,47)
Platelets (N/μL)	531666.67±23190.04 (588333,3±44378,42)	545000.0±22598.0 (637500.0±2500.0) (*)	491250.0±17132.62 (495000.0±66583,28)	503333.3±34801.02 (600000±58291.5)	643333.3±69841.09 (705000±50000)
Hemoglobin (g/L)	131.87±1.65 (134,6±6,06)	140.75±1.29 (136,5±5,5)	131.88±2.7 (163,3±10,13) (*)	142.3±1.45 (162.3±1.61) (*)	147.37±6.39 (161,3±1,76) (*)
Red Blood Cells ($\times 10^{12}$/μL)	4.77±0.15 (5,92±0,13) (*)	5.4±0.31 (3,13±0,1) (*)	5.85±0.22 (6,56±0,18) (*)	5.84±0.35 (6.47±1.125)	6.33±2.6 (6,38±1,9)

*— $p<0.05$

eters, it can be concluded that the studied complex $Cu(II)_2(3,5\text{-}DIPS)_4(H_2O)_3$ has significant therapeutic properties in case of burns.

Based on the results obtained, it can be assumed that the newly synthesized complex $Cu(II)_2(3,5\text{-}DIPS)_4(H_2O)_3$ has therapeutic properties for burn injuries. The results of this study allow us to continue researching new compounds with effective radioprotective and therapeutic properties with the expectation of discovering new highly effective compounds.

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THE EFFECT OF PIR-20 COMPOUND ON COGNITIVE DEFICIT REDUCTION IN EXPERIMENTAL GLOBAL CEREBRAL ISCHEMIA IN RATS

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ABSTRACT — A study was conducted to assess the effect of a new pyrimidine derivative PIR-20 (50 mg/kg) on the development of cognitive deficits in the conditions of global cerebral ischemia in rats. The study was performed on 40 male Wistar rats weighing 200–220 g, divided into 4 groups of 10 individuals. False-operated rats and negative control animals were injected with a suspension of purified water and tween-80, the third group of animals received Cavinton (3,2 mg/kg), the fourth — PIR-20 (50 mg/kg). All test subjects were injected intraperitoneally for ten days prior to surgery. The number of dives increased to 100%, while the decision-making time decreased by 55,2% ($p<0,05$) in the extrapolation escape test against the background of the PIR-20 compound administration. 75% of the animals treated with PIR-20 did not re-visit the dark compartment, and the time of entering the dark chamber increased by 172,9% ($p<0,05$) as compared to the group of negative control rats in the test of passive avoidance of the avesir environment. It was confirmed that the studied compound PIR-20 contributes to the improvement of cognitive and mnestic functions, which is confirmed by the results of tests of passive and active aversive environment avoidance. The obtained effect exceeded the results of the control group and the reference drug Cavinton.

KEYWORDS — brain ischemia, cognitive deficits, mnestic deficits, derivatives of pyrimidine, Cavinton.

INTRODUCTION

Cerebral stroke ranks first among the causes of disability in most developed countries as a result of the development of severe neurological and cognitive impairments [1]. Violation of cognitive and mnestic functions is most often one of the major causes ofa high level of disability [2]. Effective correction of disruptions of the memory trace with cerebroprotective drugs is of undoubted interest for this pathology. Some pyrimidine derivatives have previously established themselves as substances that improve motor and behavioral activity against the background of global brain ischemia[3,4], which was the purpose of this study.

Objective:

To study the effect of PIR-20 compound on cognitive deficit reductionin experimental global cerebral ischemia in rats.

MATERIALS AND METHODS

The study was conducted in accordance with the "Guidelines for Preclinical Trials of Drug Products" ed. by A.N. Mironov (a 2012 edition) [5]. The animals were maintained in compliance with current best practices and standards of care in laboratory animals. The experiment was performed on 40 male Wistar rats m=200–220 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-20 (50 mg/kg), synthesized at the department of organic chemistry of the Pyatigorsk Medical and Pharmaceutical Institute — branch of Volgograd State Medical University[7]. Global brain ischemia was simulated in the second and subsequent groups by bilateral occlusion of the common carotid arteries (under chloral hydrate anesthesia 350 mg/kg) [8, 9]. All objects were injected intraperitoneally for ten days before the operation. 72 hours before the simulation of irreversible occlusion of the common carotid arteries, the animals were trained in the tests of passive (the conditioned passive avoidance re ex – CPAR) and active (extrapolation discharge test-EDT) aversive environment avoidance. A day after the operation, the preservation of the memorial trace was checked. 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

Ligation of the common carotid arteries in negative control rats caused the phenomenon of retrograde amnesia, which was manifested in an increase in the number of visits to the dark compartment in the CPAR test and a decrease in the latent period of entry [10]. Repeated entries into the dark compartment were not observed in the falsely operated (FO) group of rats. The number of the animals in the negative control (NC) group that visited the dark chamber was 75%, and the time of the entry relative to the data of the experiment increased minimally (from 28,1±2,3 to 35,3±4,5) (Fig. 1). The numbers of rats receiving reference preparation Cavintonwho revisited the dark compartment of the CPAR was 50%. At the same time, the time of the arrival of the group of rats treated with Cavinton exceeded the value of the NC group by 57,8% (p<0,05). In the group of rats that received the intraperitoneal compound PIR-20, the minimum number of visits to the dark compartment by animals was observed — 25%. The approach time relative to the baseline data increased by 172,9% (p<0,05) in animals treated with PIR-20, which was statistically significant relative to the NC group of rats. In addition, the latent period of visiting the dark chamber by rats against the background of receiving PIR-20 was statistically significantly higher than that of the reference drug Cavinton by 39,1% (p<0,05).

Only 25% of the animals in the negative control group coped with the extrapolation disposal test and the diving time decreased by only 9,3% relative to the data before ischemia (58,4±4,6 sec.), while all the falsely operated subjects again completed the task and the latent period for decision-making decreased by 85,8% from the outcome (57,6±3,8 sec.) (Fig. 2). Of the group of animals that received Cavinton, 60% of subjects did not repeat the extrapolation test, and the time to solve the problem was reduced by 23,5% compared to the data before the operation (58,8±4,6 sec.). All rats treated with the experimental compound PIR-20 intraperitoneally performed the extrapolation disposal test. The latent time to make a decision about the data before the experiment (58,7±4,6) decreased by 55,2% (p<0,05). The diving time in the rats treated with PIR-20 was significantly lower by 50,4% (p<0,05) and 41,6% (p<0,05) compared to the control group and the animals treated with Cavinton.

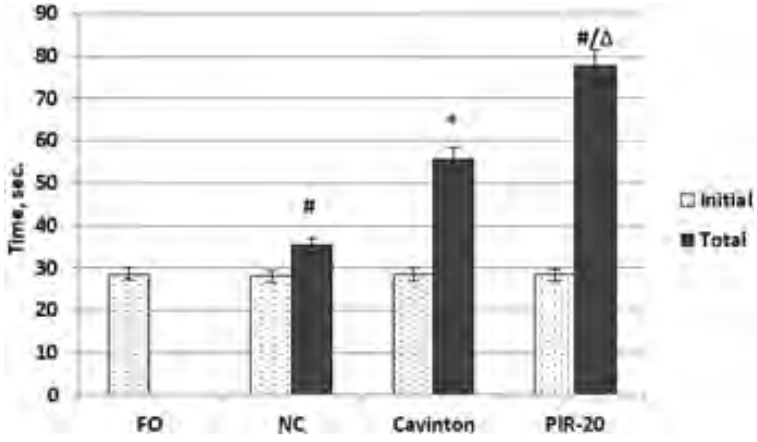

Fig. 1. *Assessment of changes in the latent period of rats entry into the dark compartment in the test of conditioned passive avoidance reflex during the intake of the compound PIR-20 and cavinton in experimental cerebral ischemia*

Note: *FO — false-operated rats ; NC — negative control rats; Cavinton — a group rats treated with Cavinton; PIR-20 — a group of rats treated with PIR-20; # — statistically significant as compared to the FO rats (p<0,05); * — statistically significant as compared to the NC rats (p<0,05); Δ — statistically significant as compared to rats treated with Cavinton (p<0,05).*

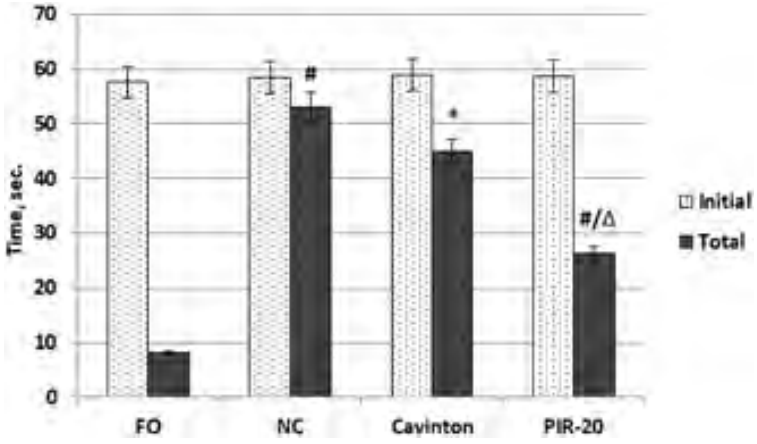

Fig. 2. *Assessment of changes in the latent period of rats' diving in the extrapolation disposal test against during the intake of compound PIR-20 and cavinton in experimental cerebral ischemia*

Note: *FO — false-operated rats ; NC — negative control rats; Cavinton — a group rats treated with Cavinton; PIR-20 — a group of rats treated with PIR-20; # — statistically significant as compared to the FO rats (p<0,05); * — statistically significant as compared to the NC rats (p<0,05); Δ — statistically significant as compared to rats treated with Cavinton (p<0,05)*

CONCLUSION

In the experimentally simulated cerebrovascular insufficiency, a pyrimidine derivative under the laboratory code PIR-20 allowed to improve cognitive and

mnestic functions in rats and showed an effect in its strength superior to the comparison drug Cavinton.

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A STUDY ON IMMEDIATE AND LONG-TERM EFFECTS OF A NOVEL MINERAL-VITAMIN COMPLEX FOR STRENGTHENING THE NAIL PLATE

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ABSTRACT — We conducted a randomized, blind, comparative, placebo-controlled, parallel study of a biologically active food supplement designed to normalize the condition of the nail plate. The paper presents the results of studying the effectiveness of this vitamin and mineral complex. It was experimentally established that a course intake of the study drug improves the appearance of the nail plate, the effect is observed in 93% of all studied cases. In respondents who used the drug, an increase in the strength of the nail plate was confirmed. It was found that a six-week course of daily intake of the product in recommended doses fully provides the nail plate with all necessary vitamins and minerals. There were no side effects during the observation period.

KEYWORDS — vitamins, mineral-vitamin complex, nail plate, strength.

INTRODUCTION

Today there are many different options for the health and appearance of nails, from traditional home recipes to professional salon procedures. The condition of the nails primarily depends on what kind of internal recharge they have, and only then on external factors [1].

Features of the structure, area and thickness of the nail depend on many factors: hormonal activity of the body, structural features of the terminal phalanx of the fingers, age, gender, and even on the profession. If the thickness of the nails is formed genetically, then their health largely depends on nutrition [2, 3]. Unfortunately, modern reality is such that, due to circumstances, a person cannot get the necessary amount of nutrients. To compensate for the deficiency of these nutrients, mineral and vitamin complexes are being developed [4].

The aim of the work was to study the effect of the use of mineral-vitamin complex on the strength of the nail plate.

The study was conducted on the basis of the Department of Pharmaceutical Technology with the course of medical biotechnology of the Pyatigorsk Medical and Pharmaceutical Institute, a branch of the Volgograd State Medical University.

OBJECTS AND RESEARCH METHODS

The object of the study was a biologically active food supplement, the certificate of state registration No.RU.77.99.88.003.E.000498.02.18 dated 02.02.2018; the approval of the Federal Research Center of Nutrition, Biotechnology and Food Safety (Moscow, Russia) No. 529 / E-1109 / b-17 dated 28.12.2017.

The vitamin-mineral complex under study is recommended as a biologically active food supplement - an additional source of vitamins: A, D3, E, B1, B2, B3, B5, B6 and minerals: zinc, iron, calcium. It promotes healthy development and growth of the nail plate.

The study included 60 volunteers aged 18 to 60 years (mean age 25.58 ± 3.1 years) who attended a beauty salon. The volunteers were divided into 2 clinical groups: the main (1st group) and control (2nd group), 30 people each (4 men and 26 women), comparable in age and condition of the nail plate. The main complaints were: rough, opaque nail plate, fragility and splitting of the nail. Slow nail growth (<0.7 mm in 2 weeks) was verified in 9 and 9 volunteers, respectively (30%). The disturbed relief of the nail plate was revealed in 33.33% and 36.36%, respectively. According to the oral survey, 60% of volunteers used gel nail polishes for a long time (more than 1 year). Some of the subjects (43.33% and 40%, respectively) had white spots and grooves on their nails, indicating a lack of zinc [5].

Volunteers received the supplement in a randomized, double-blind, comparative, placebo-controlled and parallel study. The duration of the study was 6 weeks. The supplementation was prescribed in the dose of one pill twice a day with food. In the control group, volunteers took one placebo pill twice a day with food. In the next 4 weeks of observation (7–10 weeks), long-term results were studied without intake of the supplementation.

Table 1. *Composition of the mineral and vitamin complex*

Active (functional) matrix			
№	Name	Content in one tablet, mg	% of the recommended daily intake
1	Vitamin B1 (as thiamine hydrochloride)	1,5	207
2	Vitamin B2 (riboflavin)	2,0	250
3	Vitamin B3 (nicotinamide)	20,0	222
4	Vitamin B5 (as calcium pantothenate)	5,0	167
5	Vitamin B6 (pyridoxine)	2,0	200
6	Vitamin A (as retinol acetate)	0,9	225
7	Vitamin E (as alpha-tocopherol)	15,0	200
8	Vitamin D3 (as Cholecalciferol)	0,005	200
9	Calcium	400,0	80
10	Iron	10,0	121–143
11	Zinc	6,0	133–167
Excipients			
№	Name	Content in one tablet, g	Content in a daily dose, g
1	microcrystalline cellulose	0,4619	0,9238
2	magnesium stearate	0,016	0,032
3	polyvinylpyrrolidone	0,0111	0,0222
4	hydroxypropyl methylcellulose	0,0267	0,0534
5	polyethylene glycol	0,00896	0,01792
6	iron oxide yellow	0,0028	0,0056
7	titanium dioxide	0,00896	0,01792
8	iron oxide red	0,00058	0,00116

RESEARCH METHODS

In the laboratory, the condition of the nail plate of the little finger of the left hand was recorded every two weeks in all the volunteers participating in the study. The outgrowing part of the nail plate was removed by the specialists of the beauty salon with the appropriate processing of the cut edge. The cut part of the nail was tested for tensile strength using a Pharmaceutical tester PJ-3. The procedure for determining the strength of the cut nail was carried out during the day, in order to avoid the loss of moisture and, accordingly, elasticity.

Statistical data processing was carried out by methods of variation statistics using parametric criteria. Continuous numerical values were expressed as mean values and their standard deviation ($X \pm \sigma$) [6, 7].

RESULTS AND DISCUSSION

At the initial stage of the research, the strength of the nail plate was determined. The initial measurement of the strength of the nail plate varied from 0.2 N/m^2 to 0.51 N/m^2, which is associated with the individual characteristics of the body (age, diet, professional activity, etc.). The study was carried out in the spring.

The first two weeks of taking the drug showed the following results. In the main group, the strength of the nail plate increased only in 41% of cases, in 7% of cases it remained unchanged, in 52% of cases, the strength continued to decrease. In the placebo group, only in 20% of cases, the strength of the nail plate increased, in 10% it remained unchanged. In 70% of cases, the strength continued to decline. On average, both groups showed a seasonal decrease in strength (Fig. 1, 2).

After next two weeks of receiving the drug, due to the accumulation of the drug, the clinical picture has changed a little in the main group. Already in 62% of cases, the strength of the nail plate increased compared to the previous period, in 1% it remained unchanged, in 37% of cases the strength slightly decreased. On average, the group showed a tendency to increase the strength of the nail plate. In the placebo group, the strength of the nail plate slightly increased in 70% of cases, it remained unchanged in 7%, and the strength continued to decrease in 23% of cases. Thus, on average, there was a slight increase in strength across the group.

Over the next two weeks of observation (5–6 weeks) in 90% of cases in the main group, the strength of the nail plate increased compared to the previous period whereas 7% remained unchanged. A decrease in strength was noted only in 3%. In the main group, the trend towards an increase in the strength of the nail plate persisted, as a positive growth rate of 0.22 was confirmed. In the control group, in 13% of cases, the strength of the nail plate slightly increased compared to the previous period, in 3% it remained unchanged, in 74% of cases, the strength continued to decrease. In this group, there was a decrease in density compared to the previous two weeks, respectively, the growth rate of the indicator was -0.01.

Within 7–8 weeks of observation already without taking the product in the main group in 86% of cases, the strength of the nail plate increased compared to the previous period, a slight decrease in strength was noted only 14%. In the placebo group, in 75% of cases, the strength of the nail plate slightly increased compared to the previous period, in 25% of cases, the strength continued to decrease.

In the next two weeks of observation (9–10 weeks) in the main group in 79% of cases, the strength

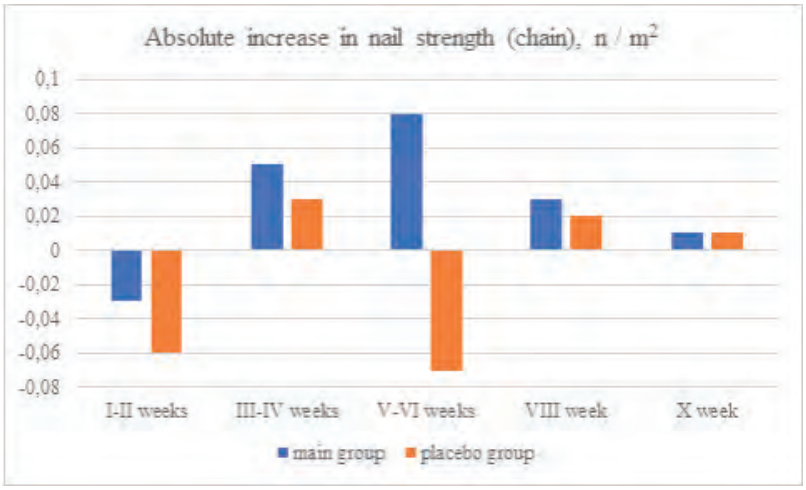

Fig. 1. *Chain strength gain of the nail plate*

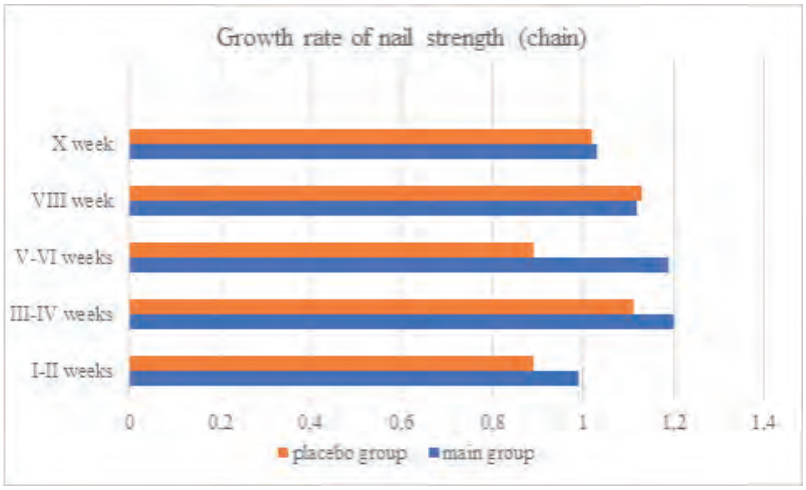

Fig. 2. *The chain growth rate of the strength of the nail plate*

of the nail plate increased in comparison with the previous period, it was noted in 21%. The average absolute increase in the indicator was 0.01±0.001 n/m^2. Average coefficient of change in strength (growth) was 1.05±0.002. On average in the group, there was a moderate trend towards an increase in the strength of the nail plate, as evidenced by a positive growth rate of 0.20.

In the placebo group, in 62% of cases, the strength of the nail plate slightly increased compared to the previous period, in 2% it remained unchanged, and in 36% of cases, the strength continued to decrease. The average absolute increase in the indicator is negative and amounts to 0.01 N/m^2 ± 0.001. Average coefficient of change (growth) of strength was 1.03±0.002. On average, the group showed a tendency towards a decrease in density compared to the previous two weeks, respectively, the rate of growth of the indicator was -0.11.

Taking the strength of the nail plate as a basis for comparison at the time of the beginning of the experiment, we obtained the following average basic characteristics for the groups (Table 2).

The given values indicate that in the main group there is an increase in the strength of the nail plate, the average strength of the plate increased by 37 ± 0.2%, there is a clear tendency to an increase in strength (the growth rate of nail strength is 0.5> 0). In the control group, the average strength of the nail plate decreased by an average of 9% ± 0.8 and there is a tendency to further decrease (-0.01 <0).

CONCLUSION

In a subjective assessment carried out by a questionnaire-survey method, the study participants before the start of therapy were not satisfied with the appearance and condition of their nails, the effectiveness of previously conducted measures to improve their appearance.

Table 2. *Average values of the basic characteristics of the time series for the first indicator*

Groups	Absolute increase in nail strength	Growth rate of nail strength	The rate of increase of strength of the nail
Main group	0,13±0,001	1,37±0,001	0,50±0,003
Control group	-0,05 ±0,001	0,91 ±0,001	-0,01 ±0,003

The results of a randomized, blind, comparative, placebo-controlled and parallel studies have confirmed the ability of the drug to increase the strength of the nail plate.

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EARLY AND LATE POSTOPERATIVE COMPLICATIONS IN PATIENTS WITH LOCALIZED AND LOCALLY ADVANCED RENAL CELL CARCINOMA

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ABSTRACT — Background: Modern Russian statistics show that the percentage of renal cell carcinoma (RCC) among all oncological diseases has increased and amounted to almost 5% in 2019. The main method of treating RCC is radical nephrectomy in localized RCC, which is supplemented by the removal of regional lymph nodes in locally advanced RCC.
Aim: To evaluate early and late postoperative complications in patients with localized and locally advanced renal cell carcinoma.
Methods: We've analyzed the results of surgical treatment and postoperative complications in 378 patients with clinically proven localized and locally developed RCC.
Results: The total number of complications after surgical treatment of patients with localized and locally advanced RCC was 24 (6.3%) patients. Moreover, in the treatment of the localized form of RCC, postoperative complications are 3 times less common than in the locally advanced form of RCC ($p < 0.05$). The most common complication after surgical treatment of RCC was bleeding which we observed in 11(2.9%) patients.
Conclusion: After surgical treatment of RCC, the proportion of complications is not high, but they can have unpleasant consequences. In the late postoperative period the most common complication bleeding is followed by lymphorrhea and urinary tract infections.

KEYWORDS — renal cell carcinoma, bleeding, postoperative complications, nephrectomy, advanced lymphadenectomy.

INTRODUCTION

Renal cell carcinoma (RCC) is one of the most important problems of oncourology. Its incidence has increased annually causing morbidity and mortality from this malignant neoplasm [1, 2]. In the structure of oncological diseases, the percentage of RCC in Russia was 4.8% in 2019 [3]. In terms of the growth rate of cancer incidence in Russia, RCC consistently ranks third (43.9%), second only to prostate and thyroid tumors [3].

The main method of treating RCC is radical nephrectomy [4]. The principles of performing radical nephrectomy include early ligation of the renal artery and vein, removal of the kidney along with the surrounding paranephral tissue, and the ipsilateral adrenal gland. The presence of RCC metastases in the lymph nodes is one of the most significant adverse factors that reduces the survival rate of patients to 5 — 30% and contributes to the risk of metastasis to distant lymph nodes. Therefore, in the early stages of development, it is necessary to perform a regional lymphadenectomy, when performing surgery for RCC. In these cases, the expansion of the volume of the operation lengthens the time of the operation, may contribute to an increase in the volume of blood loss and the development of postoperative complications.

Aim:

To evaluate early and late complications after surgical treatment of patients with localized and locally developed renal cell carcinoma.

MATERIALS AND METHODS

The study included 378 patients with proven clinically localized and locally advanced RCC (according to ultrasound and computed tomography). We did not include patients with distant metastases in the study.

Depending on the type of surgical treatment, all patients were divided into two groups:

Group 1: patients underwent radical nephrectomy without extended lymphadenectomy (n = 126);

Group 2: patients underwent radical nephrectomy with extended lymphadenectomy (n = 252).

In all patients, we've recorded the number and severity of complications that developed in the postoperative period: bleeding, lymphorrhea, a urinary tract infection, etc.

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

Both groups were dominated by patients with clinically localized RCC and kidney tumor size from 4

cm to 7 cm (cT1b): in the first group — 58 (46%), in the second group — 91 (36.1%). In the second group, there were significantly more patients with a kidney tumor size greater than 7 cm (cT2) compared to the first group: 62 (24.6%) versus 23 (18.2%), respectively ($p<0.05$). There were also significantly more patients with signs of the tumor leaving the kidney capsule and entering the paranephral tissue (T3a) in group 2: 71 (28.1%) vs. 13 (10.3%) ($p<0.05$). The distribution of patients in both groups, taking into account the clinical stage of the disease, is shown in Fig. 1.

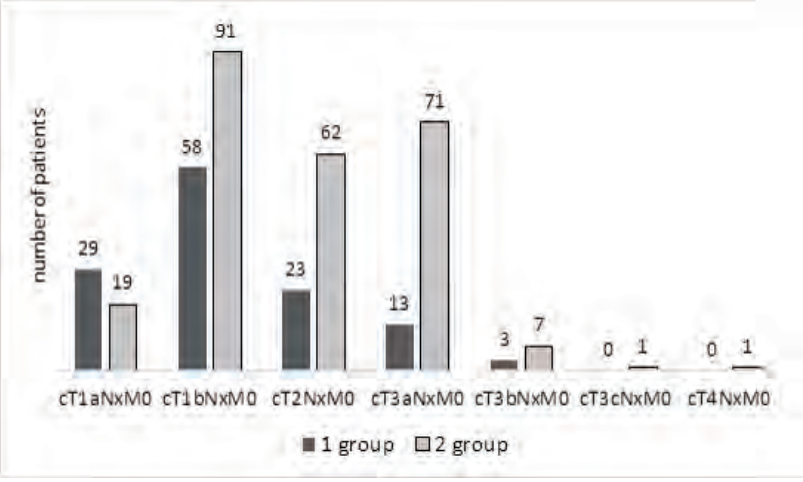

Fig. 1. *Distribution of patients in both groups, taking into account a clinical stage of the disease*

The duration of the operation in patients of the second group was significantly higher lasting 171.3±46.9 minutes, and in the NE group without regional LAE — 142.4±39.3 minutes ($p<0.01$).

We have recorded complications after surgical treatment of patients with localized and locally advanced RCC in 24 (6.3%) patients (Fig. 2). Among the patients of the first group of the study, complications were in 6 (4.7%) patients, against 18 (7.1%) people in the second group ($p<0.05$). Of all the complications, we most often encountered bleeding (both during surgery and in the early postoperative period): in 3 (2.8%) people from the first group and in 8 (3.1%) — from the second ($p<0.05$). In 10 (2.6%), bleeding was stopped conservatively. Only 1 (0.4%) patient from the second group needed emergency surgery to correct hemostasis.

In the late postoperative period, we have recorded complications in 7 (1.8%) patients. During this period, we most often encountered long-term lymphorrhea.

DISCUSSION

The standard of treatment for localized and locally advanced RCC is radical nephrectormia [4]. However, RCC up to 4 cm in size (corresponding to T1a) has a 5% risk of death within 5 years without treatment [5]. Therefore, doctors are increasingly using advanced lymphadenectomy to improve the results of RCC treatment. The addition of extended lymphadenectomy to radical nephrectomy improves the 10-year survival rate of patients with metastatic lymph node involvement. In locally advanced RCC, lymph node dissection is performed both to accurately determine the stage of the disease, and to reduce the frequency of local recurrence and increase the survival rate of patients. However, against the background of the expansion of the volume of surgery in the treatment of RCC, complications may develop in the early and late postoperative period. Our study showed that one or another complication occurs in every 16 patients with surgical treatment of RCC. Moreover, in the treatment of a localized form of RCC, postoperative complications are 3 times less common compared to the locally common form of RCC. According to Wongvittavas N et al. postoperative complications in the surgical treatment of RCC can reach 31% [6].

Bleeding is one of the most dangerous complications of radical nephrectomy. Bleeding often develops when performing an extended lymphadenectomy. This is more often associated with damage to the branches of the aorta and inferior vena cava, lumbar and adrenal vessels. In our study, 11 (2.9%) patients developed bleeding.

CONCLUSION

After surgical treatment of RCC, the most common complication is bleeding. In the late postoperative period, lymphorrhea and urinal tract infections are most frequent.

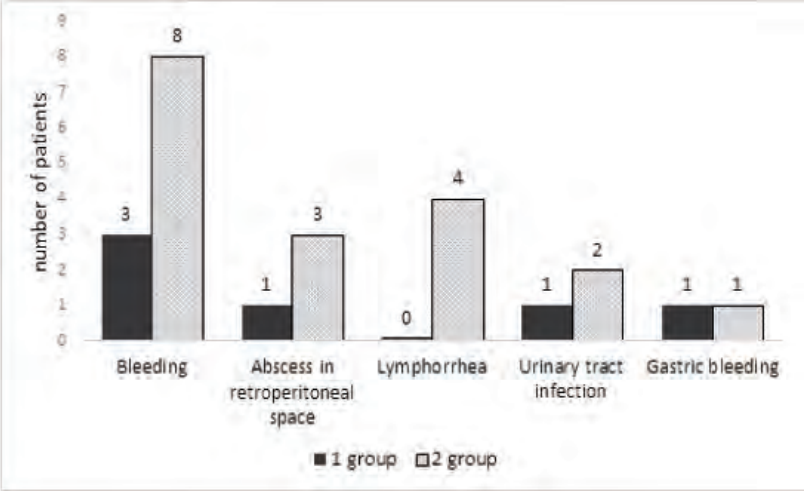

Fig.2. *Distribution of patients in both groups, taking into account complications that have developed after surgical treatment*

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LEFT VENTRICLE REARRANGEMENT IN PATIENTS WITH CRITICAL OSTIAL STENOSIS OF THE LEFT MAIN TRUNK COMPLICATED WITH ISCHEMIC MITRAL REGURGITATION

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ABSTRACT — In this work, we studied the possibility of stenting the left coronary artery trunk in patients with subacute myocardial infarction on a background of critical stenosis of the left coronary artery trunk, reduced left ventricular ejection fraction and surgically significant mitral regurgitation. We analyzed treatment outcomes of 97 patients who underwent stenting of left main trunk for subacute stage of myocardial infarction. All patients had mitral insufficiency of II–III degree. The echocardiography data were analyzed before surgery, 7 days, 30 days and 12 months after surgical treatment. Direct stenting of the left coronary artery trunk was performed in 38 (39.2%) patients. 59 (60.8%) patients underwent Culotte bifurcation stenting. A good treatment effect was achieved in 89 (91.2%) patients. As a result of the LV myocardial remodeling, EF increased by 1.44 times ($p < 0.05$). There was a significant decrease in the degree of mitral insufficiency by 39% to surgically insignificant from 5.9 to 3.6 mm ($p < 0.05$). Stent placement as an initial treatment in elderly patients with more than 24 hours after the onset of acute myocardial infarction (AMI) originated from a critical lesion of the left coronary artery trunk and concomitant pronounced mitral insufficiency proved as fully justified and safe. We assume that this treatment enables to improve ejection fraction and reduce severity of mitral regurgitation in elderly AMI patients.

KEYWORDS — myocardial remodeling, coronary stenting, left main trunk stenosis, mitral regurgitation (MR), myocardial infarction (MI), echocardiography.

RELEVANCE

At the moment, a combination of left main trunk stenosis and surgically significant mitral insufficiency is increasingly occurring in the world. Most often, patients with a combination of such pathologies show a decrease in the contractility of the left ventricular (LV) myocardium due to its hibernation [1]. A gradual decrease in pumping function leads to an increase in the volumes of the left parts of the heart, dilation of the mitral ring, violation of the closure of the valve leaflets and the appearance of a jet of regurgitation. Often, this whole process is accompanied by compensatory tachycardia, which only worsens the general condition of the patient [2]. In the organization of planned surgical treatment, preference is given to thoracic aortic coronary bypass surgery with prosthetics or mitral valve repair. If a significant atherosclerotic lesion of the first portion of the right coronary artery accompanies the pathology, the above described changes are further aggravated. However, if patients are admitted in the hospital with obvious changes in ECG interpreted as acute myocardial infarction, then in this case, majority of surgeons choose a wait-and-see attitude, due to the high risks of surgical treatment. The presence of *flabby* myocardium in acute coronary syndrome up to 6–8 hours often leads to the need to plan intraoperative LV remodeling, which poses a number of challenges: a high risk of cutting the myocardium with suture, the inability to suture the source of bleeding from the myocardium, the use of hemostatic sponges and other devices. All this contributes to an increase in the duration of assisted circulation (AC), the development of post-perfusion syndrome, which, on a background of reduced LV pumping function, can lead to severe complications, up to the impossibility of disconnecting the AC apparatus. The use of the stenting technique on the left coronary artery trunk shows good results and is increasingly used in such situations, especially against the background of the use of additional hemodynamics support devices, such as extracorporeal membrane oxygenation and intra-aortic balloon counterpulsion [3, 4, 5].

If patients with a myocardial infarction are admitted in hospital later than 24 hours after MI onset, in this case, a choice of treatment is quite disputable. Many authors argue the necessity of emergent surgical revascularization which can be fatal for the patient. Cardiac surgeons prefer delayed execution of thoracic aorta coronary bypass surgery with prosthetics or

mitral valve plastics after 3–4 weeks. Often (in 48% of cases) patients do not survive to this, or decompensation of physical condition occurs, which is a contraindication to the operation [6]. The use of isolated stenting is often also not considered, due to the high risks and high material costs for the clinic.

However, the implementation of stenting against the background of the application of various methods of hemodynamic support should be considered and studied.

Purpose of the work

is to study the possibility of stenting the left coronary artery trunk in patients with subacute myocardial infarction in the setting of critical stenosis of the left coronary artery trunk, a reduced LV ejection fraction and surgically significant mitral regurgitation.

MATERIALS AND METHODS

We studied medical histories of patients over past 8 years (2012–2020) who underwent stenting of the left coronary artery trunk on a background of subacute stage of LV myocardial infarction. (Fig. 1, Fig. 2).

Fig. 1. *Critical lesion of the mouth of the left coronary artery trunk*

An analysis of the treatment results in 97 patients (the median age was 72 ± 4.7 years), was performed. The echocardiography showed in all patients mitral insufficiency of II–III degree (mean v.c. 5.9 ± 0.3 mm), the average LV ejection fraction (LVEF) was 36 ± 2.1%. There were 62 men and 35 women. In all patients, the average surgical time after the onset of a heart attack was 72 ± 12 hours. Intra-aortic balloon

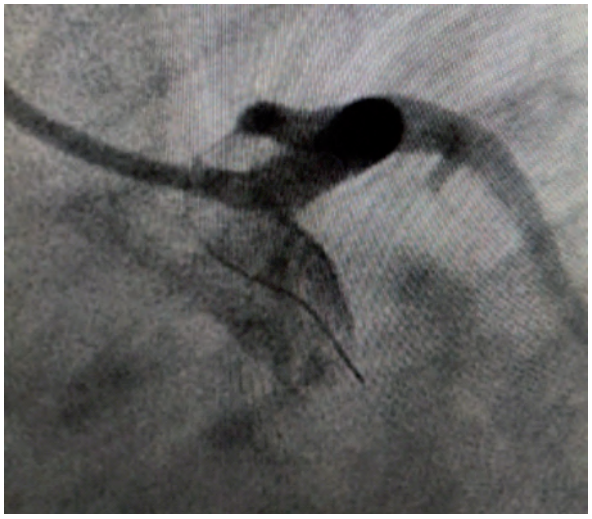

Fig. 2. *The result of stenting for left main trunk stenosis*

counterpulsation was applied in 23 (23.7%) patients; 17 (17.5%) patients had simdax infusion after surgical treatment. 62 (63.9%) patients were stented in the setting of developing cardiogenic shock. 28 (28.9%) patients had a concomitant tachysystolic form of atrial fibrillation. The average systolic blood pressure before the intervention was 86 ± 7.5 mm Hg. In 82 (84.5%) patients, large scar changes in the LV myocardium were previously identified.

An analysis of echocardiography data was performed: LVEF dynamics, LV sizes — end-diastolic volume (EDV), end-systolic volume (ESV), end-diastolic size (EDS), end-systolic size (ESS) in the time frame: before surgery, 7 days, 30 days and 12 months after surgical treatment. Efficacy was estimated with a 15% or more reduction in EDV, 15% or more ESV, and a reduction in regurgitation jet size (v.c. by more than 1.5 mm).

The received results statistically processed by means of the Statistica 10.0 program (StatSoftInc., the USA), defining a median (Me), and interquartile scope (Q1-Q3). The statistical significance of differences between groups was assessed using the non-parametric Mann-Whitney test. Values were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

Direct stenting of the left main trunk was performed in 38 (39.2%) patients. 59 (60.8%) patients underwent Culotte bifurcation stenting. Drug-coated stents of zotarolimus were used. In 46 (47.4%) patients, stenting of the right coronary artery was additionally performed. The average number of implantable stents in the patient was 2.1 ± 0.3.

Table 1. *Echocardiographic data*

Indicator	Statistical indicator	Before treatment	After 5 days	After 30 days	After 12 months
end-diastolic size, cm	M±σ Me [Q1-Q3] p	6,87±0,42	5,51±0,31 0,003	5,32±0,22 0,005	5,43±0,21 0,005
end-systolic size, cm	M±σ Me [Q1-Q3] p	4,93±0,32	4,16±0,14 0,005	4,22±0,12 0,005	4,12±0,09 0,005
end-systolic volume, ml	M±σ Me [Q1-Q3] p	155±18	92±16 0,0001	89±12 0,0001	88±16 0,0001
end-diastolic volume, ml	M±σ Me [Q1-Q3] P	199±16	151±18 0,0005	146±15 мл 0,0005	139±14 0,0001
LVEF, %	M±σ Me [Q1-Q3] P	32,6±4,1	46,1±3,6 0,0005	47,4±1,4 0,0005	45,2±3,3 0,0005

Note: *p <0.05 — the differences are statistically significant compared to the indicator before the installation of intra-aortic balloon counterpulsation*

The table below shows initial and final data of echocardiographic results after performed surgical treatment. There was a statistically significant decrease in EDS by 19.8%, ESS (by 16.1%), ESV (by 40.6%), EDV (by 24.2%). As a result of the LV myocardial remodeling, EF increased by 1.44 times ($p < 0.05$). There was a significant decrease in the degree of mitral insufficiency by 39% to surgically insignificant from 5.9 to 3.6 mm ($p < 0.05$).

The desired treatment effect was achieved in 89 (91.2%) patients. 8 (8.8%) patients died within 5 days after surgical treatment due to severe heart failure.

CONCLUSION

In elderly AMI patients hospitalized later than 24 hours after onset of AMI with an underlying critical lesion of the left coronary artery trunk and concomitant pronounced mitral insufficiency, the use of stenting as the first stage of treatment is fully justified and safe. Such treatment tactics, especially in combination with modern hemodynamic support methods (simdax, intra-aortic balloon counterpulsion, extracorporeal membrane oxygenation), leads to a statistically significant increase in the ejection fraction and a decrease in the degree of mitral insufficiency.

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ARTERIAL STIFFNESS AND CENTRAL AORTAL PRESSURE AS SIGNIFICANT PREDICTORS OF COMPLICATIONS AFTER OFF-PUMP CORONARY ARTERY BYPASS GRAFTING

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ABSTRACT — Some studies have shown that an increase in arterial stiffness and central aortic pressure increase the risk of postoperative complications for off-pump coronary artery bypass grafting (off-pump CABG).
AIM: To determine the significance of arterial stiffness and central aortic pressure indicators for predicting acute kidney injury (AKI) and cardiac events in patients after off-pump CABG.
MATERIALS AND METHODS: An analysis of off-pump CABG outcomes was carried out in 196 patients (mean age 62.7 ± 5.3 years) without clinical signs of chronic kidney disease. The patients were divided into a test (n = 56) and control (n = 140) groups, depending on the presence or absence of AKI, acute coronary syndrome (ACS), or cardiac arrhythmias in the early postoperative period. Pulse wave velocity indicators (PWV) were processed on the carotid-femoral (cfPWV) and brachio-ankle (baPWV) segments, and the results of measuring central aortic pressure (CAP).
RESULTS: It has been proven that the incidence of AKI is significantly higher in patients with higher cfPWV (n <0.001), baPWV (n = 0.034) and systolic aortic pressure (SAP) (n = 0.013). ACS was more often observed in patients with higher SAP (n <0.001) and diastolic (DAP) (n = 0.005) aortic pressure, and cardiac arrhythmias in patients with higher SAP and office systolic pressure (n < 0.001). Based on the results of logistic regression analysis, it was determined (by regression coefficients — B) that an increase in cfPWV by 1 m/s increases the risk of AKI by 85.0%. An increase in SAP by one unit significantly increases the risk of ACS by 111.0% and cardiac arrhythmias by 58.0%. It was shown that the measurement of CAP has a greater diagnostic value than the office measurement of blood pressure.
CONCLUSION: Arterial stiffness is an independent predictor of AKI for off-pump CABG. A decrease in PWV value is accompanied by a decrease in the incidence of AKI. CAP indices have a more significant prognostic value with respect to the likelihood of cardiac events after an off-pump CABG than the data of measurement of blood pressure.

KEYWORDS — arterial stiffness, central aortic pressure, acute kidney injury, acute coronary syndrome, off-pump coronary artery bypass grafting.

INTRODUCTION

Coronary artery bypass grafting (CABG) applied for multivessel disease in patients with coronary heart disease, is still one of the most common methods of myocardial revascularization [1, 2]. However, it is known that this highly traumatic intervention is associated with a high risk of postoperative complications [3]. Their prediction is difficult in patients with comparable demographic indicators and comorbid backgrounds.

To exclude the risk of perioperative complications associated with cardiopulmonary bypass used for on-pump CABG, in patients with preserved cardiac reserve, it is recommended to perform CABG on the beating heart (off-pump CABG) [1, 3]. But even such tactics cannot completely eliminate the risk of life-threatening complications in the early postoperative period.

It is difficult to predict cardiac events (acute coronary syndrome (ACS) or arrhythmias (HR)) or acute kidney injury (AKI) based on the results of standard preoperative examination of patients with coronary artery disease (CHD). However, a number of studies have shown that this category of adverse effects was often associated with impaired organ perfusion in patients with increased central aortic pressure (CAP) and arterial stiffness (AF) [4, 5, 6]. And these indicators with a sufficient degree of reliability can be assessed by non-invasive methods and do not require significant labor costs [7, 8, 9].

In clinical practice, arterial stiffness is assessed in terms of pulse wave velocity (PWV) in elastic (carotid-femoral PWV (cfPWV)) and muscle segments (brachial-ankle PWV (baPWV)) of the arterial bed [10] It has been proven that the cfPWV index, the measurement of which is considered the *gold standard*, is an independent predictor of postoperative complications [8, 9, 10].

MATERIALS AND METHODS

In the period from January 2018 to December 2020 a prospective controlled study was conducted at the clinic of the Department of Anesthesia and Resuscitation (Volgograd Medical University, Russia).

The results of planned off-pump CABG for coronary artery disease in patients with multivessel coronary artery disease were analyzed. All patients gave informed consent for participating in the study and publishing the results under assurance of confidentiality. The study was approved by the Volgograd Regional Independent Ethics Committee (IRB 00005839 IORG 0004900 [ref: 109/2017/12/07]).

Inclusion criteria:

1. Patients over 18 years of age, of both sexes scheduled to off-pump CABG;
2. Presence of a valid study of CAP and arterial stiffness.

Exclusion criteria:

1. Patients who underwent on-pump CABG;
2. Placement of a shunt;
3. Patients with permanent atrial fibrillation (AF), chronic kidney disease (CKD) stages 3–5, obesity II–III degree and mental disorders.

A total of 237 patients who met the inclusion criteria were analyzed. 41 patients were excluded from the study, who had one or more signs that met the exclusion criteria: 11 patients were diagnosed with permanent AF, 9 with CKD stage 3 (glomerular filtration rate (GFR) less than 60 ml/min/1.73 m^2), in 26 — obesity of II–III degree (BMI ≥ 35.0 kg/m^2), in 5 — one shunt was imposed, in 2 patients due to clinical necessity (unstable hemodynamics) on-pump CABG was performed. Thus, the outcomes of surgical treatment in 196 patients were finally processed.

The primary endpoint was death, clinical signs of AKI, acute coronary syndrome (ACS) or arrhythmia (AF, paroxysmal ventricular tachycardia (PVT)).

All patients were divided into the main and control groups. The main group (n = 56) included patients in whom studied complications (AKI, ACS, or clinically significant arrhythmia (ARR)) were diagnosed in the early postoperative period. The control group (n = 140) included patients without these complications. The main group was subdivided into subgroups: AKI-subgroup (n = 28), ACS-subgroup (n = 12) and ARR-subgroup (n = 25). In 9 patients, the postoperative period was complicated by the development of AKI and persistent cardiac arrhythmias, which accounts for their inclusion both in the AKI-subgroup and the ARR-subgroup for a univariate analysis.

In all patients, a planned preoperative examination was performed including the assessment of anthropometric parameters, the data of standard laboratory (including lipid, carbohydrate and protein metabolism, biomarkers of myocardial damage) and instrumental studies (ECG, echocardiography, coronary angiography, ultrasonography of the carotid arteries, assessment of the function of external respiration, etc.).

In the morning, in a state of physical and mental rest, each patient underwent 3-fold (to ensure data validity) measurements of cfPWV and baPWV using the Vicorder system (Skidmore Industries, UK). The indicators of CAP (systolic aortic (SAP), diastolic aortic (DAP) and pulse aortic pressure (PAP)) were determined after daily monitoring of blood pressure.

During off-pump CABG, all patients received general anesthesia (total intravenous anesthesia + inhalation anesthesia + narcotic analgesics with or without thoracic epidural analgesia).

Acute kidney injury was established with an increase in blood plasma creatinine by 2 times or more from the initial values and a urine output level of less than 0.5 ml/kg/h for 12 hours or more (2–3 degree AKI according to KDIGO criteria [11]). When diagnosing acute coronary syndrome (ACS), we took into account the appearance or expansion of zones of hypokinesis (according to echocardiography), an increase in ischemic changes on an electrocardiogram, an increase in the level of serum markers of myocardial necrosis (troponin-T). Arrhythmia episodes were taken into account if they lasted 24 hours or more and required medical correction.

Statistical processing of the obtained results using Statistica 10 software (StatSoft Inc., USA), after checking for normal distribution by the Shapiro-Wilk test, was performed using parametric (Student's t-test for unrelated groups) with normal distribution and nonparametric tests (U-test Mann-Whitney, Fisher's exact test) when deviating from the normal distribution. To identify independent predictors of AKI and cardiac events, multiple regression analysis was used with the determination of β-regression coefficients (B). The difference between the compared groups of variables of more than 95% ($p < 0.05$) was considered a statistically significant difference.

RESULTS AND DISCUSSION

In total, more than 50 variables were analyzed in 196 patients enrolled in the study during the entire period of hospitalization. Clinical and demographic data are presented in Table 1. None of the patients had a history of CKD or a permanent form of cardiac arrhythmias, all were in a stable condition and underwent planned preoperative preparation.

As shown in Table 1, the majority of those operated were men (83.7% of men versus 16.3% of women) and the average age of patients exceeded 60 years (62.4 ± 5.4 years). There was a fairly significant number of patients with overweight (25.0–29.9 kg/m^2) and

Table 1. *Clinical and demographic parameters*

Parameters	Study groups		P
	Main group (n=56)	Control group (n = 140)	
Age (mean±SD)	61.6±5,7	62.9±4,8	0.093
Sex (F / M)	9/47	23/117	0.569
BMI (mean±SD)	28.3 (27.5-30.2)	28.1 (25.1-29.1)	0.189
GFR, ml / min / 1.73m^2 (mean±SD)	85.9±9.1	87.4±8.3	0.364
Operation duration (mean±SD)	280.1±69.6	265.0±66.7	0.125
General anesthesia:			
Without TEA, n (%)	46 (82.1)	109 (77.9)	0.323
With TEA, n (%)	10 (17.9)	31 (22.1)	
Comorbidities:			
Hypertension, n (%)	32 (57.1)	81 (57.9)	0.526
DM, n (%)	19 (33.9)	41 (29.3)	0.318
COPD, n (%)	6 (10.7)	13 (9.3)	0.472
CCI, n (%)	14 (25.0)	27 (19.3)	0.241

Note: *BMI — Body mass index; TEA — Thoracic epidural analgesia: GFR — Glomerular filtration rate; DM — Diabetes mellitus; COPD — Chronic obstructive pulmonary disease; CCI — Chronic cerebral ischemia; SD — Standard deviation.*

obesity of the 1st degree (30.0–34.9 kg/m^2) (68.9% and 16.8% of observations, respectively), which somewhat prolonged operative duration due to the difficulty of surgical entry. Average off-pump CABG for patients included in the study was estimated as 273.1 ± 65.3 minutes.

An unfavorable comorbid status was observed in 100% of patients. Arterial hypertension was diagnosed in 57.7% of patients, type 2 diabetes mellitus in 30.6%, chronic obstructive pulmonary disease in 9.7%, and chronic cerebral ischemia in 20.9% of patients. Comparison groups for the studied parameters were comparable (p> 0.05).

Comparative analysis of PWV, CAP and blood pressure (BP) in patients of the study subgroups is presented in Tables 2, 3 and 4. In patients in whom the early postoperative period was complicated by AKI (Table 2), in the preoperative period, there were higher indicators of arterial stiffness in elastic and muscle segments of the arterial bed, as indicated by significantly higher values of cfPWV and baPWV (13.4 (13.3: 14.0) versus 8.9 (7.6; 10.6) m/s (p < 0.001) and 15.1 (14.6; 15.9) versus 10.1 (9.2; 11.9) m/s (p = 0.034), respectively). Higher levels of CAP and BP were found in patients with AKI, but a statistically significant difference was found only in SAP (131 (128; 142) versus 119 (110; 123) mm Hg, p = 0.013).

Comparative analysis of the data obtained from patients with ACS and without it (Table 3) revealed that significant differences were revealed only in terms of CAP. In patients with ACS, the SAP and DAP values were significantly higher (132 (127; 142) versus 120 (118; 129) mm Hg (p < 0.001) and 88 (83; 93) versus 79 (76: 83) mm Hg (p = 0.005), respectively) than in patients without ACS. Arterial stiffness and blood pressure were comparable.

Clinically significant arrhythmias were found in patients who had higher rates of CAP in the preoperative period (Table 4). In patients of this category, significantly higher indicators of SAP were revealed (139 (134: 142) versus 119 (110; 123) mm Hg, p <0.001) than in patients without this postoperative complication. Indicators of arterial stiffness and blood pressure did not significantly affect the incidence of arrhythmia.

It is recognized by many researchers that the use of multivariate tests can lead to an increase in the risk of type I error, but they allow to perform the modeling process and reveal the power of each predictor in relation to an increase in the risk of an adverse event.

Table 5 shows the results of the logistic regression analysis of the identified predictors of AKI and cardiovascular events. The most significant predictor of AKI was pulse wave velocity (cfPWV), with an increase of 1 m/s; the risk of developing this complication increased by 85.0%. The CAP and BP data did not have a significant prognostic value for AKI.

When predicting cardiac events, it is advisable to focus on the indicators of CAP. Hence, an increase in SAP by 10 mm Hg significantly increase the risk of ACS by 111.0%, and the risk of developing clinically significant arrhythmias by 44.0% of cases.

Table 2. *Comparative analysis of the parameters of PWV, central aortic pressure and blood pressure in patients with and without acute kidney injury (AKI)*

Parameters	AKI Me(IQR), n=28	No AKI Me(IQR), n=168	P
cfPWV, m / s	13.4 (13.3: 14.0)	8.9 (7.6; 10.6)	<0.001*
baPWV, m / s	15.1 (14.6; 15.9)	10.1 (9.2; 11.9)	0.034*
SAP, mm Hg	131 (128; 142)	119 (110; 123)	0.013*
DAP, mm Hg	87 (84,5; 95)	79,5 (71; 82)	0.078
PAP, mm Hg	44 (36; 45)	39,5 (36; 40,5)	0.746
SBP, mm Hg	135 (122; 141)	128 (119; 136)	0.067
DBP, mm Hg	85 (74: 93)	79 (72; 84)	0.071
PBP, mm Hg	50 (45; 56)	49 (45; 54)	0.518

Note: **P<0.05, statistically significant; cfPWV — carotid-femoral PWV; baPWV — brachial-ankle PWV; SAP, DAP and PAP — systolic, diastolic and pulse aortic pressure, respectively; SBP, DBP and PBP — systolic, diastolic and pulse blood pressure, respectively.*

Table 3. *Comparative analysis of the parameters of PWV, central aortic pressure and blood pressure in patients with and without acute coronary syndrome (ACS)*

Parameters	ACS Me(IQR), n=12	No ACS Me(IQR), n=184	P
cfPWV, m / s	10.0 (9.4; 12.0)	9.7 (9.3; 11.5)	0.491
baPWV, m / s	12.1 (9.9; 12.9)	10.1 (9.4; 12.5)	0.207
SAP, mm Hg	132 (127; 142)	120 (118; 129)	<0.001*
DAP, mm Hg	88 (83; 93)	79 (76: 83)	0.005*
PAP, mm Hg	45 (38; 48)	41 (34; 43)	0.116
SBP, mm Hg	133 (132; 141)	129 (125; 140)	0.801
DBP, mm Hg	86 (83; 92)	78 (73: 88)	0.746
PBP, mm Hg	47 (45; 53)	51 (47: 54)	0.440

Table 4. *Comparative analysis of the parameters of PWV, central aortic pressure and blood pressure in patients with and without cardiac arrhythmias (ARR)*

Parameters	ARR Me(IQR), n=25	No ARR Me(IQR), n=171	P
cfPWV, m / s	10.6 (9.1; 12.6)	9.1 (7.9; 10.8)	0.198
baPWV, m / s	11.4 (10.2; 14.4)	10.2 (9.4; 12.2)	0.597
SAP, mm Hg	139 (134:142)	119 (110; 123)	<0.001*
DAP, mm Hg	88 (80; 91)	80 (71; 82)	0.305
PAP, mm Hg	48 (41; 59)	37 (36; 42)	<0.001*
SBP, mm Hg	132 (131; 139)	127 (116; 134)	0.112
DBP, mm Hg	80 (77; 84)	78 (70; 84)	0.142
PBP, mm Hg	52 (50; 58)	49 (45; 52)	0.251

AKI and adverse cardiac events are the most common complications of off-pump CABG that require preventive measures.

This is one of the few studies investigating the relationship between arterial stiffness and AKI and between CAP and cardiac events after planned off-pump CABG. However, there are a number of studies confirming the relationship between increased PWV and renal dysfunction [4, 8]. So, S.A. Greenwood et al (2019) proved in their work that an increase in PWV is an independent risk factor for AKI after CABG [4]. Despite this, there are studies that describe an insignificant correlation between arterial stiffness and AKI [12]. But our study is larger than the sample size of E.

Table 5. *Indicators of logistic regression analysis of predictors of acute kidney injury, acute coronary syndrome and cardiac arrhythmias*

Complication	Predictor	B	SE	Exp(B)	95% CI		P
AKI	cfPWV	0.107	0.021	0.846	0.396	0.916	<0.001*
	baPWV	0.042	0.012	0.092	-0.119	0.491	0.635
ACS	SAP	0.021	0.003	1.113	0.085	0.293	<0.001*
	DAP	0.009	0.008	0.353	0.300	-0.997	0.181
	PAP	0.090	0.006	0.153	0.160	0.184	0.004*
ARR	SAP	0.015	0.001	0.557	0.444	0.818	<0.001*
	DAP	0.006	0.002	0.167	-0.168	2.709	0.090
	PAP	0.006	0.003	0.079	-0.546	2.718	0.301

Note: *SE — standard error; Exp(B) — regression coefficient; CI — confidence interval.*

Kidher et al. (2014). We have demonstrated that an increase in the risk of AKI is observed with an increase of 1 m/s PWV both in the elastic and in the muscular segment of the arterial bed (by 66.0% and 31.0%, respectively), which may be associated with a violation perfusion and barotrauma of the renal glomeruli [13].

There are many studies devoted to determining the relationship between the indicators of CAP and the risk of AKI and cardiovascular complications [6, 8, 9, 14], but there are not enough works that would determine the significance of these indicators in the perioperative period. Some studies have identified a close direct correlation between CAP and AKI [15], but in our study we did not find such a relationship, possibly due to the small sample size.

It is known that CAP, like arterial stiffness, is an independent predictor of cardiovascular events [8, 15]. We have proved that the CAP indicators are superior to the results of traditional blood pressure measurements in terms of the accuracy of ACS prediction. The expediency of using indicators of CAP, and not blood pressure, is also proved by other authors in their works [10]. And only in relation to the risk of postoperative arrhythmias, the use of CAP and standard blood pressure did we show a comparable prognostic value.

In our work, like many other authors, we recommend using preoperative monitoring of arterial stiffness and CAP in patients who are planned to undergo off-pump CABG in order to predict the development of AKI and adverse cardiac events. Moreover, it does not require significant material or labor costs.

Some limitations must be recognized. Firstly, the results of this study are based on a relatively small sample and, secondly, the data were processed only in patients with a low risk of AKI or cardiac events, who had CABG without cardiopulmonary bypass. Therefore, for widespread introduction of the obtained results into clinical practice, it is necessary to confirm them in large multicenter studies.

CONCLUSION

Arterial stiffness is an independent predictor of AKI in off-pump CABG. A decrease in PWV indicators is accompanied by a decrease in the incidence of AKI. Indicators of CAP have a more significant prognostic value than data of blood pressure in relation to the likelihood of cardiac events after off-pump CABG.

Conflict of interests

The authors state that they have no conflicts of interests.

Contributors

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

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RISK FACTORS FOR MALIGNANCY OF PAPILLOMAS IN THE OROPHARYNGEAL MUCOUS MEMBRANE ASSOCIATED WITH HUMAN PAPILLOMAVIRUS (HPV)

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ABSTRACT — Analysis of the HPV positive papilloma structures at different levels of pathological process development was carried out. Classical staining of preparations with hematoxylin and eosin was used for immunohistochemical determination of Ki67-positive cells and phenotyping of CD positive cells. We found that the process of papilloma formation begins with a local increase in the proliferative activity of keratinocytes which contributes to the formation of a local epithelial convex above the surface of the oropharyngeal mucosa (OPM) in the form of papilloma.
The connective tissue of the OPM adjacent to the epithelium grows at the second stage. At the same time here is a decrease in immunocytes/macrophages number of the oral epithelium not only in the growth zone of the papilloma but in the adjacent neoplasm tissue.
The third stage is characterized by the destruction of the basal membrane of the OPM. Apoptotic cells in the cambial layer and forming leukocytes infiltrate the OPM lamina propria. Virus-infected keratinocytes are phagocytized by macrophages or exfoliated from the surface of the epithelial layer. The emerging in the middle layers defect of tissue of the epithelial layer and the absence of Langerhans cells indicate a relationship between the migration of antigen-presenting cells expressing CD68 with impaired differentiation and specialization of keratinocytes.
The conclusion is based on the analogy of "leukemic failure" in leukemia and on the absence of differentiating epithelial cells between the cambium and the specialized surface layer in the long-existing papilloma. The disappearance from the epithelium of CD68-positive cells specific to the epithelial layer is a prognostic sign of malignization in the mucous membrane of the oropharynx.

KEYWORDS — HPV; HPV-associated oral epithelial dysplasia; oral mucosa, CD cells, malignant neoplasms, papilloma, risk factors for malignant neoplasms, oropharyngeal mucosa.

INTRODUCTION

More than 150 HPV subtypes have been identified In individuals with persistent HPV infection [1]. Oral precancerous lesions (OPL) and changes in the mucous membrane remain unresolved due to the etiopathogenetic complex of HPV infection depending on the virus strains and the background of patients' immunity [3, 7]. In particular, human papillomavirus lesions are known as possible risk factors or cofactors for malignant neoplasms; however, most of the worldwide research is devoted to the diagnosis of HPV strains, their prevalence and possible oncopathogenesis [9]. Anyway, the role of local immune homeostasis (LIH) of the oral mucosa (OPM) in HPV infection remains unresolved as well as the degree of pathological changes depending on LIH of the OPM and predicting the recurrence of HPV infection. Human papillomavirus (HPV) has a significant tropism for epithelial and mucosal tissues and can be found in several anatomical regions of the oral cavity [5, 8]. An increase in the number of somatic and endocrine pathologies, an unfavorable environmental situation and pandemics that reduce the immune protection of the barrier structures of the integumentary tissues in the human body lead to an increase of pathogenicity of HPV infection in mucous membranes, particularly, the oropharynx [6]. Located in anatomically significant places, papilloma can lead to substantial dysfunctions and therefore its removal requires not only conservative treatment but also surgical removal [2]. Despite numerous studies, the reasons for the spontaneous regression of neoplasms on the mucous membrane of the oropharynx related to HPV infection are not proper investigated well so far and the mechanisms of their spread and progression to malignancy indicate the necessity for research in this direction [4].

Aim of the research:
to establish morphological criteria estimating the risk of malignancy for papillomas located at the mucous membrane of the oropharynx. In this regard, the following objectives were achieved:

1. To establish most character and diagnostically significant phenotypes in the composition of cell ensembles of papilloma structures.
2. To determine the morphological prognostic criteria indicating the possibility of spontaneous recovery or the transformation of papilloma into a chronic form during the HPV infection.
3. To establish the stages of papilloma formation.

MATERIAL AND METHODS

Biopsy material of papillomas located on the mucous membrane of the oropharynx was examined in 58 patients. The distribution of material by the topography of papillomas, age groups and duration of clinical manifestations of human papillomavirus infection is shown in Table 1.

Table 1. *Distribution of clinical material of papilloma depending on age and localization*

Age groups (years old)	Localization for extraction of the biopsy specimens from the mucous membrane at:				
	paranasal sinuses	mouth	nasal cavity	pharynx	vocal cords
20–39	4	6	5	7	3
40–59	3	3	1	4	5
60 and more	2	4	4	3	4
Total	9	13	10	14	12
Total examined	58				

125 biopsies of the oropharyngeal mucosa from 58 patients were studied, obtained in accordance with fundamental ethical principles Declaration of Helsinki, GCP Rules (Good Clinical Practice) and approved by the ethical commission of Far Eastern Federal University, (Vladivostok, Russia). Classical staining of preparations with hematoxylin and eosin was used for immunohistochemical determination of Ki67-positive cells and phenotyping of CD effector cells according to DAKO protocols, PCR diagnostics to identify HPV etiology of papillomas. The analysis of the preparation and the production of illustrations were carried out using an Olympus Bx52 microscope and Olympus DP25 digital camera with original software.

RESULTS

All cases of HPV-positive dysplasia had a clinically specific visualized macro picture and histological appearance. Macroscopically, it has the shape of hemispheres of different sizes, sometimes reaching 6 mm in height. The edges of papillomas could not be clearly defined. Epithelial eminences could pass into tissues with signs of infiltration along the edges, but more often, had clear boundaries (Fig. 1).

We noted that structural features of papillomas correlate with the duration of the disease recorded in the patient's history. With a short period of the disease and doctor visits at earlier stage uneven thickenings prevail due to the keratinized layer of epithelial cells in the structure of papilloma. The basal membrane is well identified, the cambial and spinous layers are located parallel to the surface of the oropharynx mucous membrane. However, keratinocytes of the granular layer can protrude into the surface of the mucous membrane of the oropharynx (Fig. 2a).

In this case, the proper plate of the OPM does not protrude into the epithelial layers, the stroma is well expressed, but together with the epithelium it does not grow above the surface (Fig. 2a, b). The forming local outgrowths of the epithelium are formed due to an increase in the regenerative potential of the cells of the spinous layer and invagination of the granular layer.

With a chronic process and the absence of treatment, the growth of the connective tissue plate along with the epithelium is observed (Fig. 2c), with the formation of sometimes significant elevations above the surface of the OPM (Fig. 2d).

The basal membrane becomes folded, poorly identified, but the cell polarity remains. Crest-like growths of the epithelium are revealed, signs of apoptosis appear in the basal and spinous layers. Not only the epithelial surface of the mucous membrane acquired tuberosity and folding, but also the relief of the basement membrane with the adjacent connective tissue changes. There is infiltration into the spinous layer of large granulocytes with eosinophilic granules in the cytoplasm. We noted that despite the proliferation of the epithelium and then the connective tissue with the formation of a papilloma rising above the surface, the proliferative activity of the epithelium decreases, and the number of cells with signs of apoptosis increases. CD-positive cells disappear from the epithelial layer and are identified in the connective tissue of the lamina propria (Fig. 3).

This indicates a decrease of the local immunity in the mucous membrane and its barrier properties and the chronicity of HPV infection.

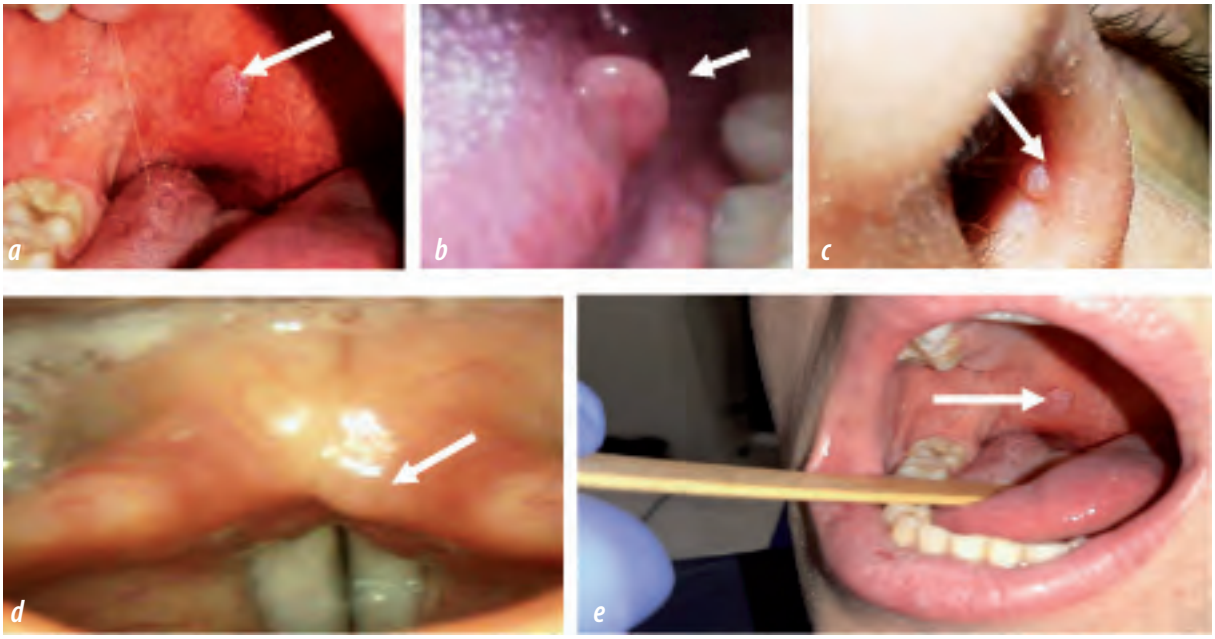

Fig 1. Papillomas of the mucous membrane: a — woman, 62 years old,palate; b — man, 45 year old,tongue c — man, 22 year old,the nasal cavity d — man 65 year old, oropharynx e — man 34 year old,palate

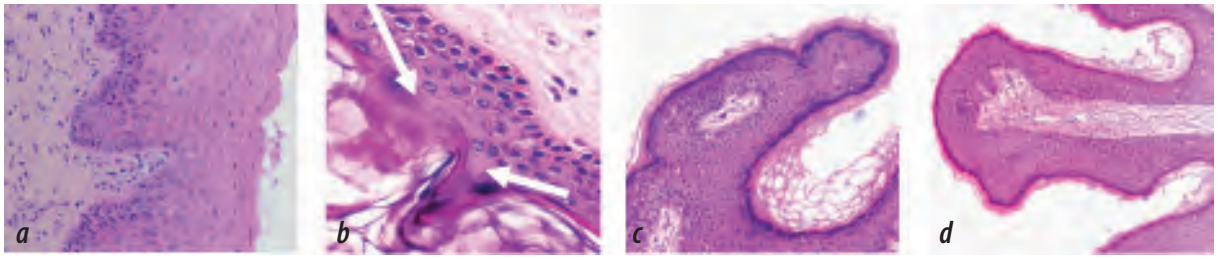

Fig. 2. a — mucous membrane in normal condition; b — 34-year-old patient with papilloma on the mucous membrane of the soft palate, which appeared 2 months later; c — 5 months later; d — 1.5 years later. Histological section of papilloma. Staining with hematoxylin and eosin. Zoom: a — ×200, b — ×400, c — ×200, d — ×200

DISCUSSION

Thus, we have established the stages of the papilloma development. The process of papilloma formation begins with a local increase in the proliferative activity of keratinocytes, which contributes to the formation of a local epithelial prominence above the surface of the mucous membrane of the oropharynx. At the second stage, the growth of the adjacent connective tissue of the OPM into the epithelial cap of the papilloma joins the process. At the third stage of the pathological process, the number of immunocytes/macrophages in the epithelial layer of OPM decreases not only in the papilloma growth zone, but also in the surrounding tissue. The fourth stage is characterized by the destruction of the basement membrane of the OPM, apoptosis of the cells of the cambial layers, and the formation of a leukocyte infiltrate. Keratinocytes with contaminated viruses are phagocytized by macrophages or desquamated onto the surface of the epithelial layer. The emerging tissue defect in the differentiating layers of the epithelium and the absence of Langerhans cells indicate the relationship between the impaired differentiation and specialization of keratinocyte differentiation cells and the migration of effector cells expressing CD68 into the connective tissue of the OPM lamina propria.

CONCLUSION

A decrease of proliferative activity, an increase in the process of apoptosis and impaired differentia-

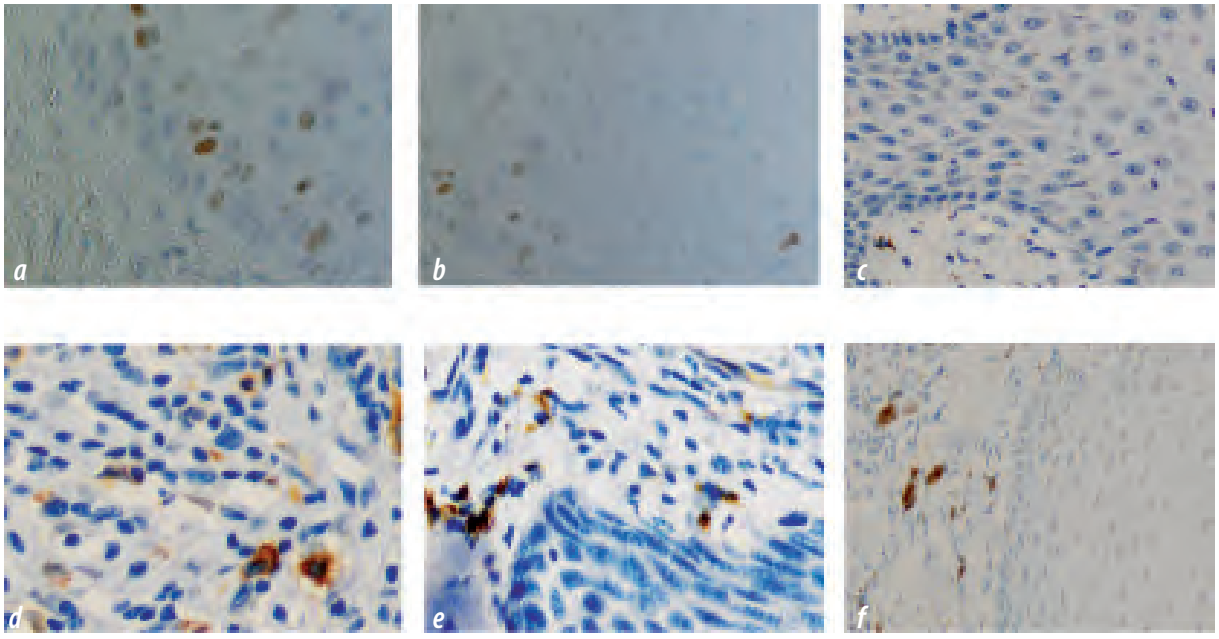

Fig. 3. *34 year old patient with papilloma on a mucous membrane of the palate. The duration of disease from the beginning of clinical manifestations: a — normal mucous membrane; b — 2 months; c — 5 months; d — 1.5 year; e, f — 2 years. Histological section of papilloma. Immune staining: a, b to identify Ki67; c, d, e, f — to identify CD68 and CD163 Immune staining. Zoom — ×400*

tion and specialization of epithelial cells resulting in a decrease of the barrier properties of the integumentary epithelium are a risk factor for carcinogenesis in the mucous membrane of the human oropharynx. The absence of differentiating layers of epithelial cells is similar to leukemic failure in leukemia.

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THE LIMITS OF EXPERT COMPETENCE AND THEIR LEGAL CONSEQUENCES IN FORENSIC EXAMINATIONS AT CIVIL TRIALS IN RUSSIA

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ABSTRACT — The article deals with the issue of determining the limits of expert competence in forensic examinations in case of improper provision of medical care in civil proceedings. The practical example shows the features of legal evaluation of expert opinion by the court in case an expert goes beyond the limits of specific knowledge. Special attention is paid to objective difficulties in differentiation of an expert legal assessment and a law-enforcement act, which arise while performing examinations on materials of medical malpractice.

KEYWORDS — competence of the expert, forensic medical examination, medical negligence, medical malpractice, iatrogenic pathology.

INTRODUCTION

A significant increase in the number of patient claims related to the quality of medical care leads to an increase in the role of forensic medical examination in civil proceedings. [1, 2] Statistical data on mortality as a result of medical errors cause alarm among health-care providers [3–5]. The cases are usually highlighted in the media, which forms a negative public attitude to unfavorable outcomes in medical care [6]. The high activity of law enforcement agencies in the investigation of potential medical malpractice cases has now led to situations when several doctors are charged with one unfavorable outcome at once.

One of the most important issues when performing such examinations is the compliance with the limits of the expert's competence. On the one hand, experts constantly face legal issues related to compliance with the requirements for the provision of health-care; standards of medical care; clinical guidelines (treatment protocols) and other regulatory legal acts. On the other hand, experts are not to make a legal assessment of guilt, illegality, etc. of legal categories. In addition, the differentiation of the field of competence of expert commission members (forensic experts and clinicians, as well as clinical specialists) in some cases can also present certain difficulties. The subject of a forensic medical examination in civil cases of damage to health while providing medical care is determined by the competence of the experts who perform it [7]. The expert's opinion is considered to be beyond the limits of the competence if an expert invades the field of non-medical sciences, such as law or psychology [8]. However, in fact, the limits of expert competence are not always obvious, as the example below clearly shows.

A patient filed a lawsuit against a hospital where he was treated with a diagnosis of *open comminuted fracture of the inner ankle of the left shin with a dislocation of the foot outwards, a multi-comminuted fracture of the fibula in the lower third, a detached fracture of the anterior-outer edge of the tibia and a rupture of the distal inter-tibial syndesmosis*. Surgical treatment performed was as follows: metallosteosynthesis. After the patient was discharged purulent fistulas appeared in the area of the ankle joint. A surgery was performed in order to remove the metal structure and a foreign body was found. It was a broken medical drill bit in the tibia. It was impossible to remove the drill bit during the surgery. A commission forensic medical examination was appointed in the case. The conclusion of this said that there was a complication during the operation in the form of damage to the drill. The nature of the injury (three-ankle Pott-Desto fracture), the presence of the drill bit in the bone (without penetration into the ankle joint cavity), the absence of contact with neurovascular formations showed that the decision of the medical council during the operation to refuse to extract the drill fragment was reasonable and correct. Performing a bone trepanation with an extraction of the foreign body from the damaged bones of the lower leg was associated with a high risk of complications that exceed the risk of complications of the operation

itself and the injury. Intraoperative damage to a surgical instrument (*a broken drill*) is not considered to be a violation of the technique and technology of the surgery and is not regarded as a defect in the provision of medical care. So it is not a subject to a forensic medical assessment of the severity of damage to health. There were no defects in the provision of medical care. The expert commission particularly noted the fact that the drill was broken at the final stage of the surgery, when almost all the elements of the metal structure were already installed. So in order to extract the drill bit, the metal structure had to be almost completely disassembled, then reassembled, which was clearly impractical in a multi-splintered fracture and associated with an unreasonably high risk, which exceeded the risk of leaving the drill bit in the bone (Fig. 1, 2).

object in his body by accident, after a control radiography was performed. So there was a violation of art. 22 of Federal Law No. 323 from 21.11.2011 "On the basics of protecting the health of citizens in the Russian Federation" — the right to be informed about the state of health. As a result of the concealment of the information about leaving of the drill in the tibia by the medical stuff the patient learned about the presence of the drill in his body by accident. This information became a complete surprise for the man and he was not properly prepared for the information of this kind. The patient had no medical education, he did not know whether it is permissible to leave a drill in the bone, what risks are associated with it, why the drill was left, etc. Health is not only the integrity of the body from a medical or legal point of view.

Fig. 1. *Metal structure and the drill fragment (indicated by arrow) in the tibia*

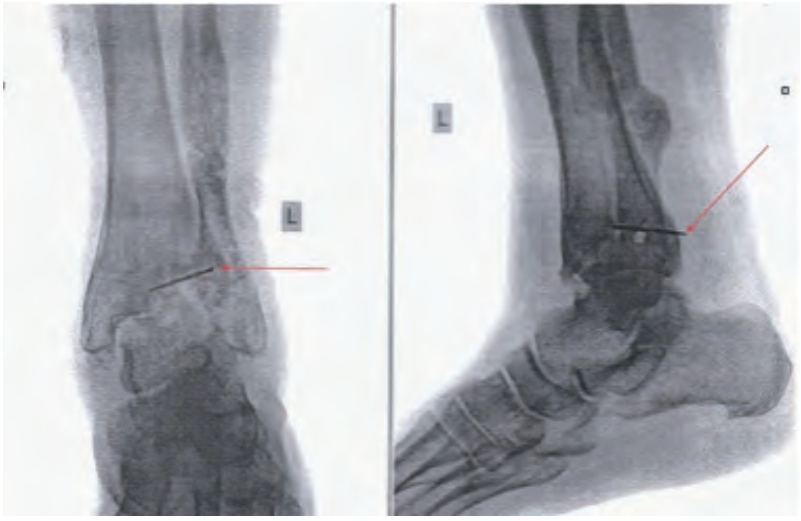

Fig. 2. *Drill fragment in the tibia (indicated by arrows) after the removal of the metal construction*

The court of the first instance refused to satisfy the claims, but the court's decision was appealed. The Court of Appeal drew attention to the fact that there were conclusions of a number of specialists in the case that contradicted the conclusion of experts, and considered this a sufficient reason for a secondary examination.

This secondary forensic examination revealed an important fact that was not taken into account during the primary examination. The patient was not informed about the complication (leaving the drill bit in the bone). The attending physician confirmed this fact in the court and said, *I did not think that it was necessary to notify the patient about this*. As a result, the patient learned about the presence of a foreign

The lack of information about the drill left in the bone caused fear and anxiety of the patient, which he experienced from the moment when he became aware of the presence of the drill in the bone. The defect in the provision of medical care was not the damage to the drill during the surgery or the decision to leave the drill in the bone, but the concealment of the information about the drill from the patient. Thus, the failure to provide the patient with information about the presence of a drill in the body was a violation of both Federal law and one of the fundamental principles of medical activity: *it is not necessary to treat the disease, but the patient*. Moreover, it is not known what the medical staff expected, hiding the fact of the presence of the drill in the bone. In any case, the patient had to

be taken to a control radiography, in which the drill would have been detected.

The court's assessment of the secondary examination report is very interesting. The court decided that the provision of incomplete information to the patient was a violation of the general rights in the field of health protection, while the medical care provided to the patient was of good quality and did not cause damage to health. The decision of the court of the first instance was completely annulled and a new decision was made, which partially satisfied the claims for compensation for non-pecuniary damage.

Assessing the conclusion of the secondary examination, the court pointed to contradictions and illogic facts in its conclusions. However, there are contradictions both in the conclusion of the expert examination and in the court's decision.

To name but a few:

— if the expert opinion was illogical and contradictory, it is not clear why the court put it as the basis for the decision;

— if the rights of the consumer were violated, but no damage to health was caused while providing medical care, then it is not completely clear what the person consumed (it is obvious that he was a consumer of medical services);

— if the rights of the patient in the field of health protection were violated, it is not clear why the court concluded that medical care of appropriate quality was provided;

— following the logic of the court, in each case of compensation for non-pecuniary damage, it is necessary to appoint a forensic psychiatric examination, since non-pecuniary damage (according to Article 151 of the Civil Code of the Russian Federation) is physical or moral suffering;

— if the court considered it necessary to conduct a forensic psychiatric examination, it is not clear why it was not appointed;

— if the court considered that any legal assessment was beyond the competence of the expert examination, then why was there a question for an expert: *What exact defects were there while providing medical care, what were they expressed in, what exact standards and norms of treatment were violated, what consequences for the health of the patient entailed*?

One thing is clear: the primary examination, which, in the opinion of the court, did not go beyond the limits of the expert competence, did not reveal any defects or violations in medical care. The court of the first instance also did not identify them, and if the secondary examination had not established the fact of improper informing the patient about the results of the surgery, the court's decision would have remained the same. During the secondary examination the patient was examined by a surgeon. The patient was fixed on painful sensations in the area of the ankle joint and was firmly convinced that they were associated with the presence of a drill in the bone, as well as the need to remove the drill from the bone. Each doctor during the diagnosis of somatic pathology evaluates the neuropsychiatric status of the patient, a psychiatric examination is not required for this. Whatever the court means by the general rights of the patient in the field of health protection, the violation of these rights would not have been established without an appropriate expert opinion, which would have led to an unfair court decision. As a rule, materialistic approach to the provision of medical care is a characteristic of healthcare providers, especially of operating surgeons. So it is surprising to see the judges' lack of understanding that a surgery is not a repair of a mechanism, but a complex act that affects both the body and the mind of the patient. We can partly agree with the court that the application of federal laws should be beyond the competence of experts. Within the framework of the examination, it is quite sufficient to assess the compliance with departmental regulatory legal acts, such as procedures and standards for the provision of medical care. However, in this particular case, the examination indicated a violation of a fundamental principle of medical practice *to treat not the disease, but the patient*. The understanding of this principle and its practical application belongs to the field of special knowledge in the area of medicine and it is within the competence of experts.

CONCLUSION

The analysis of this example from expert practice allows us to draw a number of conclusions:

1. Despite the traditional view that any legal assessment is outside the competence of the judicial examination, the courts are not always able to make such an assessment on their own.

2. Expert legal assessment of the quality of medical care, if it is sufficiently justified and factually correct, contributes to a proper consideration and resolution of civil law disputes in the field of healthcare and medical services, even if the experts go beyond the limits of their competence. Knowledge in the field of medical law can be attributed to the competence of forensic medical examination.

3. When conducting commission and complex forensic medical examinations on the materials in potential medical malpractice cases, experts should not limit their work reviewing only medical documentation. It is important to analyze all the materials of the case, including the testimony of medical staff in court sessions.

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SPECIFIC FEATURES OF VARIANT ANATOMY AND MORPHOMETRIC CHARACTERISTICS OF THE PALATAL VAULT IN ADULTS WITH DIFFERENT GNATHIC AND DENTAL TYPES OF ARCHES

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ABSTRACT — Cone-beam computed tomograms of 68 people (age — 21–35) with physiological occlusion and various gnathic dental arches were analyzed by a method developed for identifying the palatal arch index, taken as a height (depth)-to-width dimension ratio. The results of the study revealed that palatal parameters are determined by main variants (types) of the palatal vault. In case of the mesopalatal type of the arch (index value — 35–45%), the width parameters exceeded the depth values by an average of 2.4 times, while the divergence angle of alveolar processes was 116.7 ± 5.6°. The dolichopalatal type of the arch (index value – above 45%) featured domination of the width parameters over the depth-related ones, by an average of 1.8 times, while the alveolar processes divergence angle made up 127.6 ± 6.1°. As far as the brachypalatal type of the arch is concerned (index value — below 35%), the width parameters exceeded the depth parameters by 4.0 times on average, the divergence angle of the alveolar processes being 113.5 ± 5.3°. The obtained data can be used in clinical orthodontics when diagnosing pathologies of the palatal vault, as well as to interpret data from additional methods of examination and to choose the right treatment for issues related to the dental arch shape and size.

KEYWORDS — dentofacial system; upper jaw; types of palatal vault, cone-beam computed tomography, hard palate, alveolar process, dental arches.

INTRODUCTION

Modern dentistry and maxillofacial surgery, as rapidly developing areas of medicine has witnessed growing importance of X-ray-based diagnostics methods. The high research and applied value of these methods, which is due to improved X-ray and computed technologies, offer a new perspective in evaluating diagnostic data of dental patients in different age. [7, 18, 20, 24, 30, 37, 48].

The constantly growing share of plastic and reconstructive interventions in maxillofacial surgery and surgical dentistry makes it relevant to study individual features of the jaw structure [5, 13, 16, 22, 36]. In this connection the upper jaw shape and morphometry variability present the basic point when dealing with orthodontic and orthopedic treatment methods [6, 19, 25, 29, 34, 40, 54]. Respective literature shows that a detailed study of the maxillary processes, firstly, is of fundamental theoretical significance, and secondly, has an applied value in identifying pathological changes of the maxillary system, in interpretating X-ray data, as well as in arranging treatment and rehabilitation for dental patients [1, 11, 23, 35, 41, 47, 53]. The palate, which is the boundary between the oral cavity and the nose, is closely related to the development of the dentoalveolar system, as well as the skull as a whole. The alveolar process in regard to its topographic location has a particular importance for clinical disciplines [2, 17, 32, 44]. The palatal vault is an anatomical structure shaped by the hard palate bones (the palatal processes of the upper jaw and the horizontal plates of the palatal bones) and the palatal parts of the alveolar processes [28, 50]. The bone palate shape and size correlate with the dental arch parameters in different directions. Besides, relationship between the size of the dental arches and the parameters of craniofacial complex is established and modern classifications for dental arch types are proposed [4, 12]. Specific features of maxillofacial parameters in view of sex dimorphism and racial differences are shown [21, 27].

Specifics of the hard palate morphology have been studied in multiple works focusing on diagnosing and treating congenital anomalies, namely, cleft palate and issues affecting alveolar processes, hard and soft palate [3, 49, 57].

The influence of the growth type for the gnathic face part and the dental arch morphometric parameters in occlusion anomalies in the sagittal and transversal directions was shown. This has a significant impact on the palatal arch configuration. Special features of tooth rotation were identified as well as their effect on the dental arch shape and parameters [31, 33, 52].

Currently, there are plenty of methods available for studying the maxillofacial area, including the palatal arch [15, 26, 51, 56]. Among the research methods, special attention is paid to such methods as X-ray cephalometry, cone-beam computed tomography, and electron microscopy [8, 14, 38].

To assess the palatal arch parameters, there is an index proposed, which is calculated as the palate depth-to-width ratio, while a digital factor of 31–32% is recommended for identifying the norm at a young age. However, in these studies there are no data on variability of shape of the dental arches, as well as the dental arch width and the apical basis have on the palatal arch parameters [10, 45]. The specific features of the palatal arch are often the factors that determine orthodontic and prosthetic tactics for treating patients with various maxillofacial pathologies [9, 39, 43, 55].

There are certain results available, revealing changes affecting the palatal arch though orthodontic treatment offered to patients with distal occlusion [42, 46]. However, the authors in the above studies showed no hard palate parameter variability for different types of dental arches, nor did they point at the severity of the alveolar process divergence in relation to the palatal part of the arch, which, in turn, was the aim of this current study.

Aim of study:

to determine specific features of the alveolar process divergence angle in people with different morphometric palatal arch parameters.

MATERIALS AND METHODS

A retrospective study was conducted, where cone-beam computed tomography (CBCT) scans of 68 patients (aged 21–35, with physiological occlusion and various gnathic, dental types of dental arches) were studied. The CBCT images were used to evaluate the palatal arch parameters at the deepest areas, usually at the level of the second premolars. The transversal dimensions were identified in three main positions. First, the dental arch width between the vestibular odontomers (tubercles) of the second premolars at the occlusal contour was measured. The second measurement was performed to identify the width of the palatal arch alveolar part. The measurement was done between the points located on the second premolar neck on the palatal side of the arch. The third measurement in the transversal direction was aimed at identifying the width of the palatal part of the arch. The measurement was performed between the points where the alveolar processes join the palatal processes of the upper jaw.

The palate height was determined from the palatal arch deepest point to the line connecting the necks of the second premolars lingual surface.

The obtained linear parameters allowed determining the palatal arch index taken as a ratio of the palate height (depth) to the width of the alveolar part. The index helped identify three groups. IN case of an index value ranging within 35% to 45%, the palatal vault was classified as *mesopalatal*, with 25 tomograms analyzed within the group.

An increase in the index pointed at a palatal arch belonging to the deep (dolichopalatal) type, where 21 tomograms were analyzed. A decrease in the index was typical of the low (brachypalatal) type, which was detected on 22 tomograms.

Apart from the *palatal arch index*, the parameters of the *palatal arch module* were calculated as half-sum of the palate height and the width of its alveolar part. Further, the alveolar process divergence angle was identified in relation to the width of the palatal part of the arch.

The statistical data processing relied on the Microsoft Excel 2013 software and the statistical SPSS Statistics (Version 22) software package. The critical level of possible null statistical hypothesis was set at 0.05.

RESULTS AND DISCUSSION

The morphometric analysis of cone-beam computed tomograms revealed that the palate parameters are determined by the major types of the palatal arch (Table 1).

The results of the study showed the specific features of the alveolar process divergence angles in people with different palatal arch types. During that, there were some differences noted in the morphometric parameters of the investigated anatomical area.

The main indicator of the palatal arch type was its index, which, with an average size, varied from 35% to 45%, which corresponded to the *mesopalatal* type. In the *dolichopalatal* type, the palate index was above 45% reaching an average of 54.84±2.76%. People with the *brachypalatal* type featured an index below 35% — 24.87±1.29% on average.

Patients with the *dolichopalatal* type of the arch had a difference of 17.7 ± 0.9 mm in the size between the transversal dimensions of the alveolar and palatal processes, which determined the divergence angle of the alveolar — 127.6 ± 6.1 degrees on average (Fig. 1).

Table 1. *Main parameters of the dental arches and the palatal arch in the studied group, (M±m), (p ≤ 0.05)*

Dental arch and palatal parameters at the level of the second premolars	Variants of palatal vault		
	deep	low	average
Dental arch width (mm)	46.4 ± 2.1	51.6 ± 2.7	45.4 ± 1.9
Palatal arch width (mm)	13.3 ± 0.7	23.3 ± 1.1	19.1 ± 0.8
Alveolar part width (mm)	31.0 ± 1.3	38.2 ± 1.6	32.8 ± 1.5
Palatal vault depth (mm)	17.0 ± 0.6	9.5 ± 0.3	13.7 ± 0.4
Palatal vault index (%)	54.84 ± 2.76	24.87 ± 1.29	40.53 ± 1.95
Palatal vault module (mm)	24.0 ± 0.9	23.85 ± 0.74	23.25 ± 0.69
Palatal vault angle (degrees)	127.6 ± 6.1	113.5 ± 5.3	116.7 ± 5.6

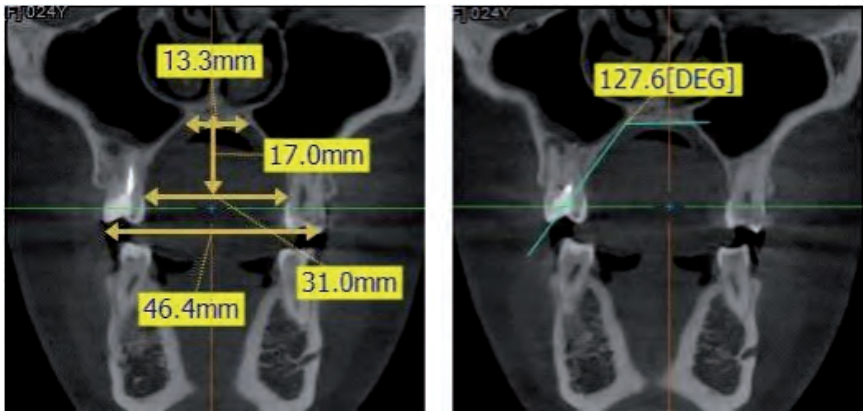

Fig. 1. *Main parameters of the dolicho-palatal arch type at the second premolars level*

The width of the palatal part of the arch in people with the *dolichopalatal* type was 13.3 ± 0.7 mm, whereas the alveolar part, at the level of the tooth necks, was 31.0 ± 1.3 mm. The depth of the palatal arch was 17.0 ± 0.6 mm, the palatal arch module being 24.0 ± 0.9 mm.

In people with the *brachypalatal* type of the palatal arch, the difference in size between the transversal dimensions of the alveolar and palatal processes was 12.9±0.5 mm, which determined the value of the alveolar process divergence angle, 113.5 ± 5.3 degrees on average (Fig. 2).

The width of the palatal part of the arch in people with *brachypalatal* type was 23.3 ± 1.1 mm, which is significantly above that in people with a deep palate ($p<0.05$).

The width of the alveolar part, at the level of the tooth necks, was 38.2 ± 1.6 mm. The depth of the palatal arch was 9.5 = 0.3 mm. At the same time, the palatal arch module was 23.85 ± 0.74 mm and featured basically no difference from the index observed in people with the dolichopalatal type of the arch ($p<0.05$).

In people featuring the *mesopalatal* type of the palatal arch, the difference in size between the transversal dimensions of the alveolar and palatal processes was 14.7±0.6 mm, which determined the divergence angle of the alveolar processes — 116.7 ± 5.6 degrees on average (Fig. 3).

The width of the arch palatal part in people with the mesopalatal type was 19.1 ± 0.8 mm, and the alveolar part, at the level of the tooth necks, was 32.8 ± 1.5 mm. The depth of the palatal arch was 13.7 ± 0.4 mm, while the palatal arch module was 23.25 ± 0.69 mm.

Given the above, identification of the alveolar process divergence angle in people with different palatal arch morphometric parameters using advanced X-ray-based methods should be a mandatory item in orthopedic dentistry and orthodontics at stages like comprehensive examination, selecting the tactics for assisting patients with dentoalveolar issues, as well as when assessing the effectiveness of dental treatment (rehabilitation).

CONCLUSION

1. The data obtained through studying cone-beam computed tomograms of patients with a complete set of permanent teeth and a physiological occlusion point at a relationship between the palatal arch morphometric parameters (height, depth) and

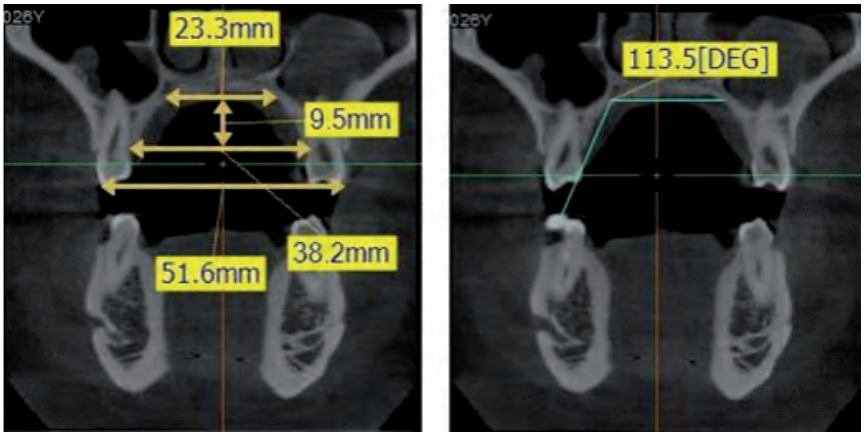

Fig. 2. *Main parameters of the brachypalatal arch type at the second premolars level*

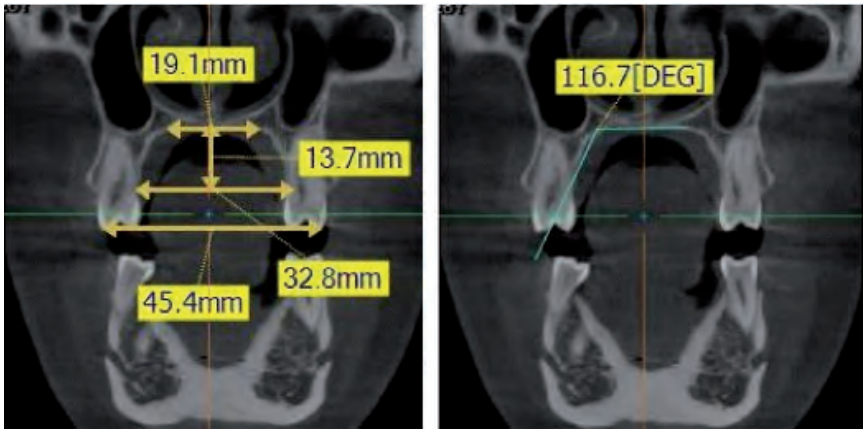

Fig. 3. *Main parameters of the mesopalatal arch type at the second premolars level*

the width-related dimension values of the dental arches. The type (brachy-, meso-, dolichopalatal) of the palatal arch is determined by index values taken as the palatal arch height (deep)-to-width ratio values.

2. For the mesopalatal arch, featuring an index varying within the range of 35–45%, which in linear dimensions points at the width parameters prevailing over the depth values by an average of 2.4 times, the difference in size between the transversal dimensions of the alveolar and palatal processes was 14.7±0.6 mm, whereas the value of the alveolar process divergence angle was 116.7 ± 5.6°.

3. In case of the dolichopalatal arch, with the index exceeding 45%, which in linear dimensions indicates the prevalence of the width parameters over the depth parameters, on average by 1.8 times, the difference in size between the transversal dimensions of the alveolar and palatal processes was 17.7±0.9 mm, the alveolar process divergence angle being 127.6 ± 6.1°.

4. In case of the brachypalatal type of the palatal arch, where the index is below 35%, meaning in linear dimensions a prevalence of the width parameters over the depth parameters by an average of 4.0 times, the difference in size between the transversal dimensions of the alveolar and palatal processes was 12.9 ± 0.5 mm, while the divergence angle of the alveolar processes was, on average, equal to 113.5 ± 5.3°.

5. The palatal arch module value, which was taken as the ratio of half-sum of its height (depth) parameters to the width of the alveolar part, in the mesopalatal type was 23.25 ± 0.69 mm, while for the dolichopalatal and brachypalatal types the values were 24.0 ± 0.9 mm and 23.85 ± 0.74 mm, respectively.

6. Cone-beam computed tomography, which features specificity, high sensitivity, and low radiation load, allows obtaining the most reliable diagnostic information concerning the cranio-facial complex bone structures. Improving the visualization algorithms, analysis of cranio-facial complex bone structures, in view of the patient's individual features, will allow standardizing methods of dental research, as well as modifying the conventionally accepted systems for the respective data analysis and interpretation, thus helping setting reliable diagnosis to patients with congeni-

tal anomalies (cleft lip, alveolar process, hard and soft palate), as well as occlusion anomalies and deformities in the sagittal and transversal directions.

7. The inclusion of the palatal arch index values in people with physiological types of occlusion and various types of dental arches in the *Clinical protocols for diagnosing and orthodontic treatment of dental anomalies in outpatient conditions* will help reduce the time spent by orthodontists on clinical examination and diagnosing, enhance the diagnosing reliability for dental issues, and improve the planning of orthodontic treatment stages for patients in their period of permanent teeth occlusion.

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THE POTENTIAL OF MICROCOMPUTED TOMOGRAPHY IN STUDYING THE VARIANT MORPHOLOGY OF THE DENTAL CANAL-ROOT SYSTEM

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ABSTRACT — High-resolution microfocus computed tomography performed on a Skyscan 1176 (Bruker) device was used to study morphological features of the canal-root systems of the upper jaw first molars in residents of the European part of Russia. An analysis of coronal, axial and sagittal sections of 134 upper jaw first permanent molars removed for medical reasons revealed variations in the canal-root system structure. In 85.8% of the cases, the first upper molars had three separate roots: two vestibular and one palatal; in 14.2% of the cases the roots make up different fusions. The palatal root bent in the buccal direction in 52.9% of the cases, while the mesiobuccal root bent in the distal direction in 87.9% of the cases. The greatest variety of curvatures was observed in the distal-buccal root: towards the mesial side — in 28.3% of the cases, another 21.6% of cases being curved towards the distal side. The palatal root in 90.3% of the cases had one canal; 9.7% of the cases featured two canals, while the distal-buccal root in 73.9% of the cases had one canal, and two canals — in 26.1% of the cases. In 89.6% of the cases, the mesiobuccal root had two canals; in 10.4% of the cases — one canal. The mesiobuccal root of the first upper molars was of an oval shape, elongated in the vestibular-oral direction, the most common structures being those of Type IV (43,3%), II (25.4%), III (8.2%) and VI (7.4%) by F. J. Vertucci. The occurrence rate of extra types of root canal structure was 7.4%.

KEYWORDS — endodont, dental canal-root system, microfocus computed tomography, root canal morphology, upper jaw first molar.

INTRODUCTION

Highly reliable, safe, non-invasive methods of X-ray diagnostics employing computer software allow identifying the patterns of individual variability in the dentoalveolar system [2, 7–11, 14, 20]. Continuous advance in the respective technologies contributes to better scientific understanding of various aspects of maxillofacial clinical anatomy, which is of interest for dentists, maxillofacial surgeons, neurosurgeons, otorhinolaryngologists, ophthalmologists and other specialists [5, 13, 22–24, 31].

Endodontics, as one of the most complex and popular field of modern dentistry, requires clinical specialists to continuously improve their professional knowledge and manual skills. In economically developed EU countries (data source: the European Society of Endodontology, 2005), successful and long-term outcome of endodontic treatment through the initial treatment is achieved in 80%, while its effectiveness implies not only complete absence of clinical symptoms, yet also by X-ray signs of a healthy status in the periapical tissues [3, 32, 35]. The following factors have an effect on the successful outcome through endodontic treatment offered of complicated cases of dental caries: the doctor's qualifications; the technologies employed for diagnostics and treatment; the degree of observing the respective standards and protocols when planning and carrying out medical and diagnostic activities; the variations in the morphological structure of the dental canal-root system [26, 29, 34].

Modern endodontic treatment is a high-tech medical procedure based on new principles of medicine, technology and ergonomics. The need to solve modern problems resulted in the development and implementation of new approaches to mechanical treatment of root canals, irrigation protocols, canal filling technologies, principles of post-endodontic dental restoration, the design of new tools, devices, medicines and filling materials, as well as changes in the endodontics terminology [27, 30].

The molars represent the most complex issue in endodontic treatment, which is accounted for by their remote location in the dental arch, significant structural variations, and frequent observed deviations in the expected number of roots and canals, all this meaning that studying the variant morphology of permanent molars is of fundamental and applied value [1, 21, 28].

In clinical practice, proper understanding of the individual features of the dental canal-root system allows the doctor to calculate the depth of proper

mechanical treatment and the force effect that can be applied to the dental hard tissues, as well as to control the impact on the tooth when moving it through orthodontic treatment [25, 33].

Given their effects of summation, subtraction and projection distortion in the configuration and size, as well as due to the obtained image being 2D, conventional methods of X-ray diagnostics (orthopantomography, radiovisiography) used in endodontics prove effective only in ⅔ of cases offering an opportunity for objective evaluation of the root canal, as well as for correlating them with the status of the periodontium, interalveolar septa and periapical bone tissue [4, 18].

The CBCT method, which features a high resolution and a low radiation stress, offers the following advantages: visualization of 3D dental images with no projection distortion; studying the 3D anatomy of the root canal system in view of individual structural variations; detecting apical pathologies and areas of disturbed integrity in the dental roots; identification of complications in endodontic therapy [12, 19].

Microcomputed tomography (Micro-CT), which allows reproducing 3D microscopic images of the examined object morphology and its inner microstructures at submicron resolution, as well as it allows identifying areas of pathological alteration in tissues, has proven its value — both research-related and applied — in medicine and in dentistry [6, 15–17]. Despite the available research data, there is no information about the variability of the canal-root system structure of the upper jaw first permanent molars in residents of the Russian Federation, which explains the aim of this study.

Aim of study: expanding the understanding of the variant morphology of the canal-root systems in the first upper jaw molars in people residing in the European part of Russia, based on data obtained through microcomputed tomography.

MATERIALS AND METHODS

The X-ray morphological studies were carried out subject to the requirements of the Local Bioethics Committee upon obtaining informed voluntary consent from all the patients. The study implied examining 134 upper jaw first molars extracted due to exacerbated chronic apical periodontitis (103 teeth — 76.9%), marginal periodontitis (21 teeth — 15.7%), and orthodontic indications (10 teeth — 7.4%). The average age of the patients was 35.8±9.7, while the gender was not taken into consideration. The inclusion criteria were: permanent erupted teeth; fully developed roots; closed apical opening; no endodontic dental treatment. The exclusion criteria included the following: external or internal resorption; sealed root canals and tooth cavities; undeveloped apex; artefacts at Micro-CT.

The following morphological features were evaluated: the number of roots; the number of root canals; the root canal configuration (direction, curvatures); fused roots. Since in the first maxillary molar the meso-buccal (MB) root has the most complex anatomical structure and rather a variable morphology, the visualization of the root canal configurations in MB has been systematized in view of F. J. Vertucci's classification (1974) (Fig. 1). Other types of root canal configurations that failed to be attributed to a certain group by F. J. Vertucci (1974) were considered not classified.

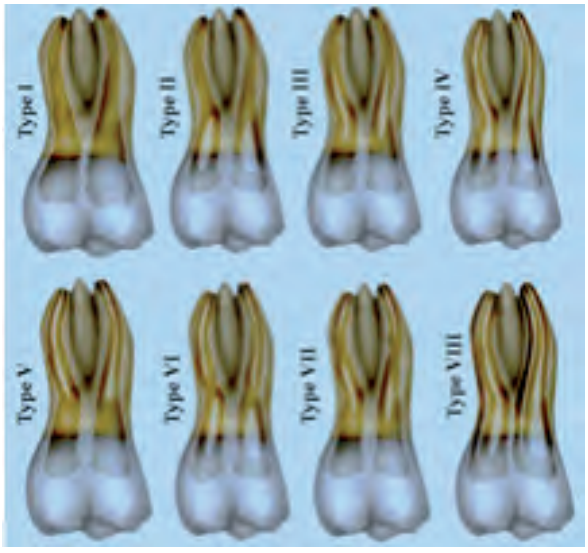

Fig. 1. *The canal configurations (Vertucci F. J., 1974): Type I (1-1) — one root canal and one apical opening; Type II (2-1) — two root canals connecting in the apical one-third part; Type III (1-2-1) — one root canal dividing into two canals, which further merge into one and exit through one apical opening; Type IV (2-2) — two separate root canals ending with two separate apical openings; Type V (1-2) — one root canal that splits as it approaches the apical opening; Type VI (2-1-2) — two root canals that join together and then separate coming closer to the apical opening; Type VII (1-2-1-2) — one root canal that divides, joins together and then opens with two apical openings; Type VIII (3-3) — three separate root canals in one root*

The architecture of the dental canal-root system was assessed through Micro-CT using the SkyScan 1176 microtomograph (Bruker, Belgium). The scanning protocol in the Skyscan 1176 (10.0.0.0, Bruker-microCT) software was as follows: X-ray voltage 90 kV, X-ray current 270 μA, filter Cu 0.1 mm, image

pixel size 8.77 μm, camera resolution setting high (4000-pixel field width), tomographic rotation 180°, rotation step 0.3. The images were reconstructed with the Nrecon (1.7.4.2) software. The spatial orientation as well as selection of individual areas was performed within the Data Viewer (1.5.6.2) software. Data visualization and analysis was performed in the CT-analyzer (1.18.4.0) software. 3D visualization of the obtained results was done with the CTvox (3.3. 0r1403) software (Fig. 2).

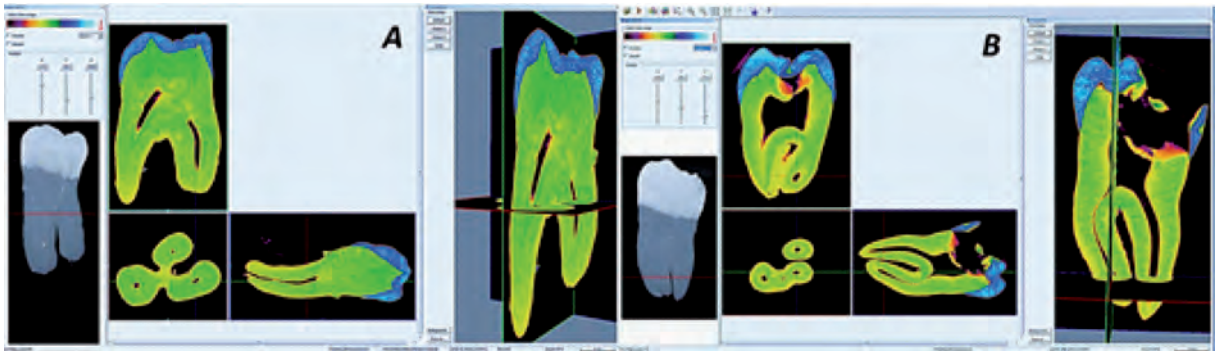

Fig. 2. *A screenshot of reconstructed 2D projections at the level of the lower third of the tooth root in the coronal, axial and sagittal projections (A — the first upper molar with separated roots; B — the first upper molar with fused roots). Pseudo-color maximum intensity (MIP) 2D projection, segmented in order to visualize the root canal morphology subject to the colorimetric optical density scale: enamel — blue-light blue color; dentine, cement — green color; canal-root system — black color*

RESULTS AND DISCUSSION

Taking into account the current data of morphological, clinical and radiological studies, the canal-root system of teeth is a complex formation, including the main canals, additional canals, lateral branches and the apical delta. The system of trunk canals in one root is represented by numerous variants, mainly due to the number and location of canals. According to scientific data, the prevalence of additional canals in different groups of teeth varies from 15 to 98%. The first maxillary molar is the largest tooth featuring a complex root morphology and a variable root-canal system. In 85.8% of cases (n=115), the first maxillary molar has three separate roots: two vestibular (MB – mesiobuccal, DB — distal-buccal) and one palatal (P). The distal-buccal root in 8.9% of cases (n=12) fuses, partially or completely, with the palatal root, while fusion of the mesiobuccal and distal-buccal roots is to be observed in 4.5% of cases (n=6), and the mesiobuccal and palatal roots — in 0.8% of cases (n=1)(Fig.3).

Table 1 shows types of root curvatures of the first maxillary molar.

The palatal root, as the longest one, was curved sharply to the palatal side, had a ribbon-shaped canal, which was wider in the mesio-distal direction, whereas 52.9% of the cases revealed a bent in the buccal direction in the apical third of the root, and in 36.6% of the cases it was straight. The palatal root had one canal in 90.3% of the cases (n=121), and two canals — in 9.7% of the cases (n=13). The significant prevalence of buccal curvature of the palatal root apex (52.9%) is the reason behind the high likelihood of a perforated wall of its root canal at instrumental processing stages. Similar data concerning the palatal root curvature can be seen in the work by J. D. Pecora (1991), who detected a palatine root bent towards the buccal side in 54.6% of the maxillary first molars. The distal-buccal root is of a conical shape and in 41.8% of the cases was straight, or curved towards the tooth axis in the mesial (28.3% of the cases) or in the distal (21.6% of the cases) direction. The distal-buccal root had one canal in 73.9% of the cases (n=99), and two canals in 26.1% of the cases (n=35). The mesiobuccal root was flattened and elongated in the vestibular-oral direction, while the apical part was bent distally in 87.9% of the cases, in another 89.6% of the cases (n=120) it had two canals, and in 10.4% (n=14) — one canal.

The upper jaw first molars erupt when there is a lack of space in the distal parts of the narrow-shaped dental arches at the age of 6–7 years. Given that, the mesiobuccal root is narrow and is elongated in the vestibular-oral direction. In the narrowest part of the fissure-shaped root canal, its mesial and distal walls join (Type IV by Vertucci — 43.3% of the cases; n=58). In the event there is no complete separation of the canals, one canal remains in the apical third of the dental root (Type II by Vertucci — 25.4% of the cases; n=34). In other cases, other structure options develop,

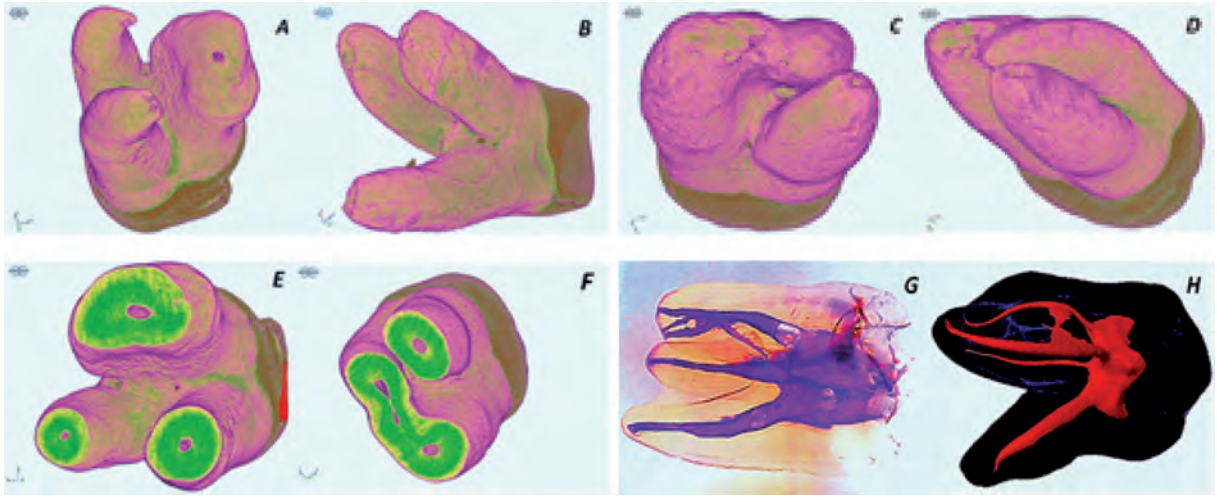

Fig. 3. *3D microtomographic images of variants of the structure of the root system of the first molar of the upper jaw: A, B — the first upper molar with three separate roots; C, D — the first upper molar with accrete roots; E — axial cut at the level of the lower third of the tooth root with separate roots; F — axial cut at the level of the lower third of the tooth root with accrete roots; G — Pseudo-color 2D maximum intensity projection for visualization of root canal configuration; H — 3D volumetric rendering for visualization of the root canal configuration*

Table 1. *Types of root curvature, first maxillary molar, (n = 134)*

Root curvature	Mesiobuccal root		Distal-buccal root		Palatal root	
	Abs., n	Rel., %	Abs., n	Rel., %	Abs., n	Rel., %
Straight	12	8.9	56	41.8	49	36.6
Curved towards cheek	1	0.8	10	7.5	71	52.9
Curved towards palate	1	0.8	1	0.8	3	2.3
Mesial curvature	2	1.6	38	28.3	7	5.3
Distal curvature	118	87.9	29	21.6	4	2.9

including complex systems of intertwining root canals with transversal anastomoses and additional canals (Table 2).

Given the results of studying the apical third of the first upper molar roots, apical openings were identified, which match the exit points of the mainline

Table 2. *Quantitative description of various root canals in the mesiobuccal root of the first upper molar, (Vertucci F. J., 1974), (n = 134)*

Channel configurations (F.J. Vertucci type)	Teeth 1.6, 2.6	
	Abs., n	Rel., %
Type I (1-1)	3	2,3
Type II (2-1)	34	25,4
Type III (1-2-1)	11	8,2
Type IV (2-2)	58	43,3
Type V (1-2)	3	2,3
Type VI (2-1-2)	10	7,4
Type VII (1-2-1-2)	4	2,9
Type VIII (3-3)	1	0,8
Additional configuration types	10	7,4

canals, following the type of root canal configuration, at a distance of no more than 5 mm from the anatomical tip of the dental root. In the mesiobuccal root of the first upper molar, extra openings of the lateral canals (apical delta) were detected in 16.4% of the cases (n=22); in the distal-buccal root — in 6.7% of the cases (n=9); in the palatal root — in 14.2% of the cases (n=19). Evaluation of the root canal types in the mesiobuccal root of the first upper molar revealed that the most common structures were those of Type IV and Type II (by F. J. Vertucci) — 43,3% and 25.4%, respectively. The remaining types accounted for 32 cases (23.9%), while Types III and VI dominated — 8.2% and 7.4%, respectively (Fig. 4).

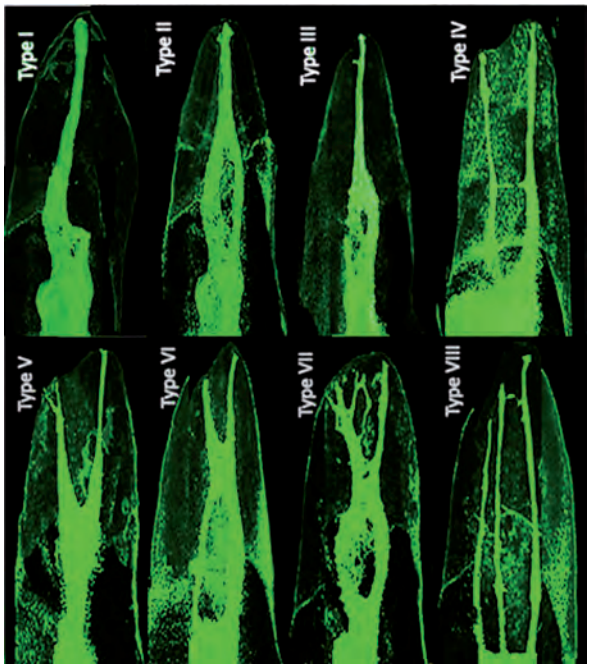

Fig. 4. *3D reconstructed images of eight types of root canal configuration in the mesio-buccal root of the first upper molar according to the Vertucci classification*

Notable is that within the studied sampling, of the mesiobuccal roots of the first upper molar, additional types of root canals were observed in 7.4% of the cases (n=10) (Fig. 5).

The obtained data enhance the clinical and X-ray clinical representation of the canal-root system in the first upper molars, as well as it indicates the diagnostic value of Micro-CT when it comes to identifying additional root canals. Intraoral radiography proved efficient in 8% of the cases only when detecting a second canal in the mesiobuccal root of the first upper molar (mesiobuccal, MB2), whereas the data of clinical studies was reliable in 50% of the cases, CBCT — in 54%, and an operating microscope — in 58% of the cases. The results obtained through Micro-CT (M. Yamada; 2011) prove that in 76.7% of the cases, the mesiobuccal root of the first upper molar contained complex configurations and extra lateral root canals, as well as apical branching.

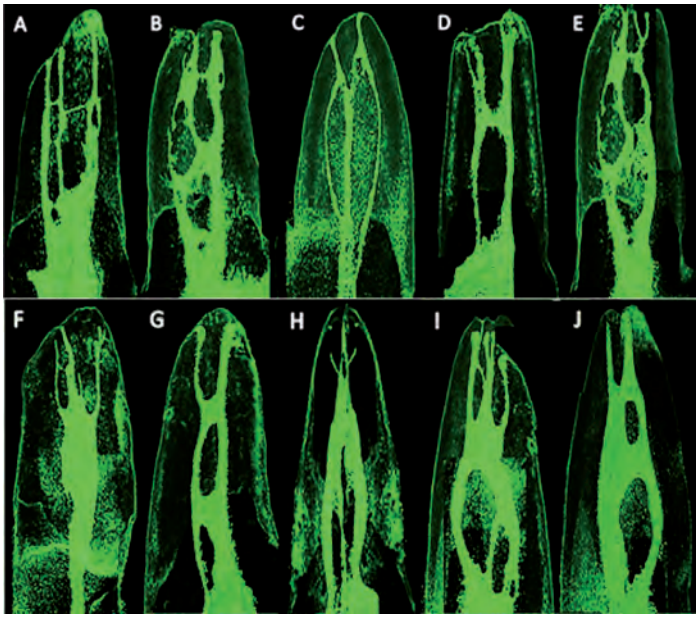

Fig. 5. *3D reconstructed images 10 additional configuration types that are not included in the Vertucci classification (A-J)*

CONCLUSION

1. Microcomputed tomography, taken as a high-tech precision method with a high resolution for visualization, allows obtaining an objective idea of the variant morphology in the dental canal-root systems, in view of quantitative and qualitative indicators with a minimum number of errors. Unlike conventionally accepted methods of X-ray diagnostics, Micro-CT offers a way to design 2D projections of maximum intensity (MIP) and 3D reconstructions of pseudo-color volumetric rendering with minimal sample preparation. Using 3D pseudo-color staining with a colorimetric optical density scale allows visualizing clearly the resorption foci in the dental canal-root systems, the degree of apical openings formation, as well as carious lesions of the tooth root part.

2. An analysis of coronal, axial and sagittal sections of 134 first permanent maxillary molars revealed variations in the canal-root system structure. In 85.8% of the cases, the first upper molars proved to have three separate roots: two vestibular (MB — mesiobuccal; DB — distal-buccal) and one palatal (P), while in

14.2% of the cases, the roots featured different types of fusion. The palatal root bent in the buccal direction in 52.9% of the cases, and the mesiobuccal root had a curvature in the distal direction in 87.9% of the cases. The greatest variety of curvatures was to be found in the distal-buccal root: towards the mesial side — in 28.3% of the cases, with another 21.6% of the cases – towards the distal side.

3. Micro-CT data revealed that in 90.3% of the cases the palatal root had one canal; in 9.7% of the cases — two canals, while the distal-buccal root in 73.9% of the cases had one canal, and in 26.1% of the cases — two canals. As for the mesiobuccal root, it had two canals in 89.6% of the cases, and in 10.4% of the cases — one canal.

4. The mesiobuccal root of the first upper molars was of oval shape, elongated in the vestibular-oral direction, with the most common types of structure belonging to Types IV (43,3%), II (25.4%), III (8.2%) and VI (7.4%) (by F. J. Vertucci). The occurrence of extra root canal configurations was 7,4%.

5. Micro-CT, as a non-invasive, highly reliable method of 3D X-ray diagnostics, given its capacity to identify a detailed microanatomy of the dental canal-root system, allows identifying the number, direction and degree of the root curvature, the root canal configuration, the degree of their patency, as well as the number and localization of secondary canals and transversal anastomoses, which is required for improving instrumental treatment and effective obturation of the dental root-canal system.

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EXOSKELETON FOR LOWER JAW FRACTURES TREATMENT

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ABSTRACT — The aim of this study was to study the possibility of the lower jaw exoskeleton as a means of treating its fractures. The analysis took into account the ability of the extrafocal osteosynthesis apparatus to maintain the spatial orientation of bone fragments under load. For this, the apparatus was analyzed by the finite element method on 3D models and a study was carried out in a special stand on the bones of the human lower jaw. As a result of the analysis, it was revealed that the lower jaw exoskeleton makes it possible to qualitatively fix the fragments of the lower jaw for the period of fracture healing.

KEYWORDS — lower jaw, fracture, application, apparatus, treatment.

INTRODUCTION

In modern maxillofacial traumatology, there is a tendency to increase the prevalence of injuries to the maxillofacial region (from 3 to 8%) [1, 2].

The nature of fractures of the lower jaw is due to the peculiarities of its anatomical structure [3–9]. In this regard, the issue of injuries of the maxillofacial region can be attributed to topical issues of modern dentistry [10], which allows us to judge the economic, social and medical significance of solving the problem of complex treatment of fractures of the lower jaw [11, 12].

We have developed and patented the device "Exoskeleton of the lower jaw" (Patent for invention No. 2655086 dated 23.05.2018). During the development of which its characteristics were given using the finite element method and bench tests (Fig. 1).

The aim of the study is to analyze the use of exoskeleton in the treatment of fractures of the lower jaw

MATERIALS AND METHODS

The study involved 36 bones of the human lower jaw, an apparatus for external fixation of the human lower jaw exoskeleton, consisting of parts of the Ilizarov apparatus.

3D bone scanning was carried out according to the author's method (application for invention No. 2020,107,207 from 19.02.2020) in the Agisoft PhotoScan program based on internal algorithms, positioning accuracy was > 99.95%.

To represent and analyze the obtained data on real objects during the development of the design of the lower jaw exoskeleton, a study on 3D models in the Solidworks mathematical analysis program for finite elements (Fig. 2) is necessary.

In order to study the stability of the fixation of the fragments of the lower jaw bones using the apparatus for external fixation of the lower jaw exoskeleton, a loading stand was created to simulate the load on the lower jaw, to which the lower jaw with the exoskeleton was fixed. For modeling, various weights weighing 2–12 kg were consistently suspended. The results were evaluated by computer analysis immediately after the study.

RESULTS AND DISCUSSION

During the experiment, almost complete immobility of bone fragments relative to each other was established at a load of 2 kg, which corresponds to the force of chewing muscles from the fracture side when treated with external fixation devices in the period after the fracture. This allows us to talk about the high quality of fixation of bone fragments for healing a bone wound at the initial stages. With a load of 7 kg, there was a displacement of bone fragments within the range of 7 kg — 0.04 ± 0.01 cm. The load of 6.5 kg corresponds to a period of 1 month after fracture of the lower jaw from the side of the fracture when treated with external fixation devices. At this point, primary stabilization of bone fragments occurs, which will prevent minimal possible displacements. By the time of 28–36 days (duration of treatment with a mandibular exoskeleton), after radiological confirmation, the external fixation apparatus is dismantled. With a load of 12 kg, which corresponds to 2–3 months after a fracture of the lower jaw, from the side of the fracture, when treated with external fixation devices, there is a divergence of bone fragments by a distance of 0.3 ± 0.05 cm. In this time period after the fracture, the patient does not use an external fixation device, the fragments of the lower jaw are fixed due to the newly formed bone connection (Fig. 3).

In the graph of the change in the width of the gap between the bone fragments, an exact coincidence of

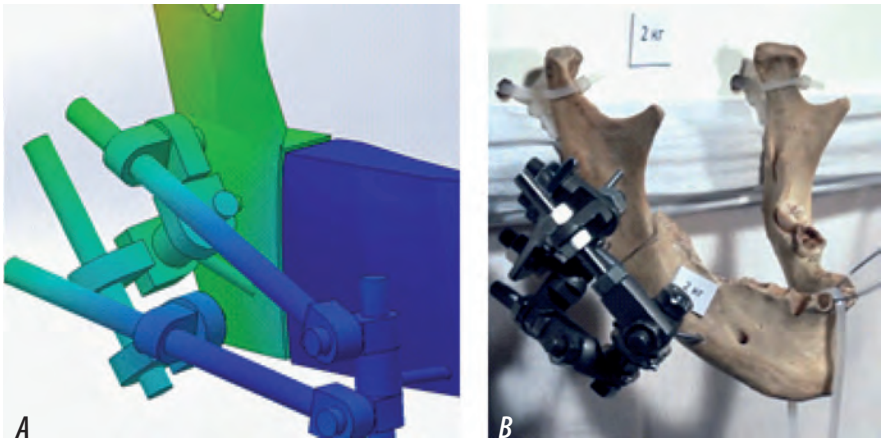

Fig. 1. *A — Finite Element Motion Analysis in Computer Environment, B — Experimental Load Test*

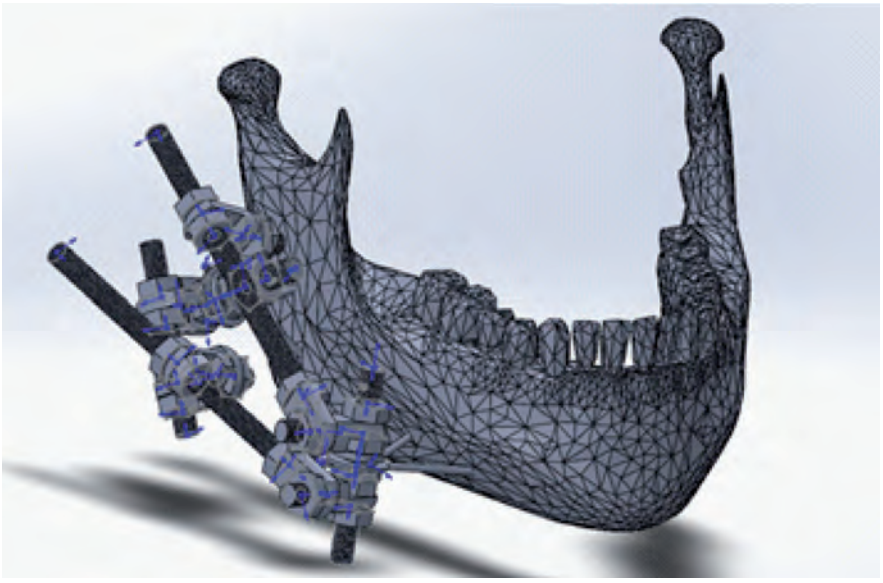

Fig. 2. *Reposition of bone fragments in Solidworks*

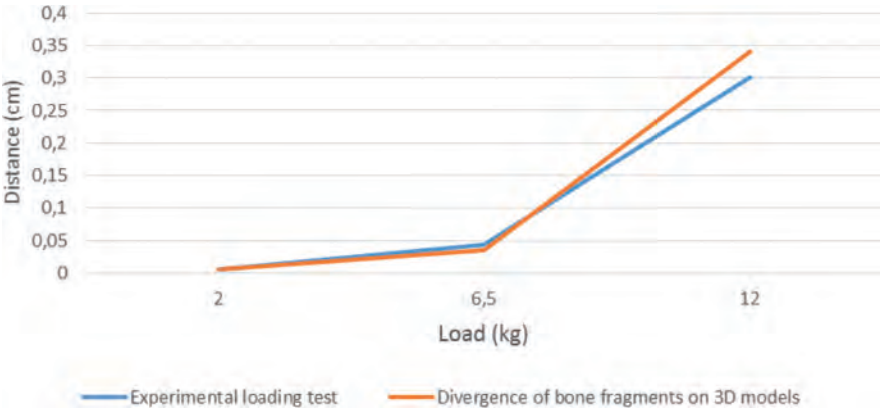

Fig. 3. *Change of slit width between bone fragments in experimental loading test*

the data of the computer simulation and the experimental load test is clearly observed, which is once again confirmed by the Student coefficient (Table 1).

CONCLUSION

Thus, we can state that the proposed apparatus for external fixation of the lower jaw exoskeleton

Table 1. *Student coefficient at different bone loads*

Change in fracture slit width when simulating fractures on lower jaw bones (kg)	Change in fracture slit width when simulating fractures on lower jaw bones (kg)	Student Coefficient Value
2	1,9	2,85
7	6,5	5,07
12	13	3,58

works in the area of maxillofacial region. It performs reposition and fixation of the bone fragments of the lower jaw under conditions of chewing functioning of the restored fracture of the lower jaw.

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COMPARATIVE CLINICAL AND RADIOLOGICAL ASSESSMENT OF ACUTE AND CHRONIC PERI-IMPLANT MUCOSITIS

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ABSTRACT — Dental implants used as artificial additional support for orthopedic structures have allowed solving more than a few issues faced by dentistry. However, complications are not always avoidable, with inflammation in peri-implant tissues (as respective literature holds it) being the most common one observed.

This paper offers a view at comparative assessment of the clinical and radiological data concerning acute and chronic peri-implant mucositis. The study included 218 patients (678 implants) who had their peri-implant tissues examined, with around 166 (24.55%) found to have soft tissue inflammation of varying severity.

Finding new effective methods of treating such complications will take a deeper differentiation of inflammatory and destructive issues affecting the soft tissues at the peri-implant area. The clinical and radiological research methods carried out through the study allowed identifying the differential signs, which serve the basis for detecting the nature of the acute and chronic course of peri-implant mucositis. During that, a low level of oral hygiene plays a negative role in this inflammation etiology.

KEYWORDS — implantation, peri-implant mucositis, oral hygiene indices, periodontal indices.

INTRODUCTION

Dental implantation is one of the most promising areas in dentistry, where, along with its positive results, there are complications to be observed. Despite the variety of advanced types of implants, as well as their installation and integration methods, inflammatory complications still remain quite relevant an issue [1, 4, 7–12]. Most often, both in the early and in the late post-implantation period, peri-implant soft tissue inflammation (mucositis) occurs; should that be the case, patients suffering from the issue need rehabilitation [2], whereas the subsequent peri-implantitis may lead to complete implant disintegration [7].

The causal relationships of peri-implant diseases still remain poorly understood, the main etiological factors including dental plaque-induced bacterial infection [3]. Experience shows that the process often turns lengthy and chronic, yet in some cases it features an acute onset. While studying the currently available literature, we failed to find a description of specific features pertaining to either chronic or acute course of peri-implant mucositis. Proper and reasonable treatment will take advanced differential diagnosis of these pathologies [5], since peri-implant mucositis is an inflammatory process affecting soft tissues [6], which is of reversible nature, and this, in turn, may help avoid further implant disintegration.

Aim of study: to study the clinical and radiological features demonstrated by patients with chronic and acute course of peri-implant mucositis, thus aiming to identify the future tactics for their treatment.

MATERIALS AND METHODS

To arrive at the aims set for the study, the data obtained through monitoring the peri-implant tissues status in the area of 678 implants (218 patients) was analyzed. The observation lasted carried out for 10 years — from 2009 to 2019. Clinical analysis of long-term outcomes revealed inflammation of varying severity in the area of 166 implants (24.5%).

The outpatient control included a clinical index evaluation of periodontal and peri-implant tissues: the Green-Vermillion index (OHJ-S); the gingival Loe-Silness index; the Muhlemann index, as well as the periodontal Russel index; the dental and implant mobility Miller-Fleszar index; the integral implant functioning factor (by M. Z. Mirgazizov). The statistical data processing was done using the SPSS 25 software package, with the values of the arithmetic mean (M) and standard deviation (SD) of quantitative features and percentage for nominal values calculated. The groups were compared through the Student, Mann-Whitney, and Pearson chi-square (χ^2) criteria with the Yates correction. The results were considered different at the statistically significant level of $p<0.05$.

RESULTS AND DISCUSSION

A detailed analysis of clinical images showing peri-implant mucositis revealed different levels of inflammation. There was a wide clinical variability of the

signs, which suggested a more detailed investigation. It helped find out that 104 cases of mucositis affecting the sites at 83 implants (79.8%) (Group I), had an inflammation of the chronic type. The patients complained of a burning sensation, slight pain in the gums when touched, bleeding gums around the implant, and some minor swelling. There was also congestive hyperemia observed, while no peri-implant pockets or discharge from the peri-implant cuff were detected. Part of the patients had never suspected the pathology and learned about it only at an outpatient checkup. The clinical image matched the nature of chronic inflammation in the soft tissues. At 21 implants (20.2%) (Group II), the degree of inflammation affecting the peri-implant zone was more severe: significant edema of the peri-implant gum, bleeding at light touch, significant hyperemia, serous or purulent discharge from the peri-implant cuff during palpation, while there was often a false peri-implant pocket developing due to the respective peri-implant soft tissue edema. There was no implant mobility observed, while the clinical presentation reflected acute inflammation in the soft tissues. Table 1 shows clinical and radiological symptoms of Groups I and II.

In order to identify distinctive clinical signs for differential diagnostics, the study implied comparative assessment of acute and chronic peri-implant mucositis clinical symptoms. Given acute mucositis, the pain in 81% of cases is significant ($p<0.001$), while chronic mucositis offered no sharp pain in 69% of cases, with a third of patients never complaining of pain. Gum bleeding, though, proved an inevitable symptom in both groups ($p=0.877$).

Gum palpation in Group II was different meaning that in 90.5% of the cases it was sharply painful ($p<0.001$), which confirmed acute inflammation, while the majority of the patients in Group I (80%) reported moderate pain, another 6% reporting none of it. The type of the discharge from the peri-implant cuff differed, too. The shares of serous discharge in the groups in question were comparable and accounted for around a third of the cases (33.7% in Group I and 28.5% — in Group II, $p=0.849$). The remaining cases, however, differed radically. In Group II, 71.4% of the cases were found to have some purulent discharge, which is a significant factor pointing at purulent inflammation. As for Group I, 66.6% of the patients had no discharge ($p<0.001$).

Given that oral hygiene is one of the major factors ensuring stable functioning of implants, we studied a number of indices pertaining to the chronic and acute course of peri-implant mucositis. Table 2 below shows the results obtained.

In case of chronic peri-implant mucositis, oral hygiene, following the Green-Vermillion and Muhlemann indices, was not good, yet only reflecting a satisfactory level; the Loe-Silness index reflected a degree of inflammation that could be described as mild, while in case of acute course, the oral hygiene was significantly worse, matching a poor level, whereas the degree of inflammation, based on the Loe-Silness index, featured a medium degree of severity. There were significant differences identified in the hygiene and periodontal status indices. For all the three indices, higher values were typical for patients with acute peri-implant mucositis ($p<0.001$).

Table 3 contains data on the periodontal status indices.

Patients with acute peri-implant mucositis featured a periodontal Russel index value that exceeded significantly that in patients with chronic mucositis ($p<0.001$). The tooth and implant mobility, though, differed neither statistically nor clinically (stable in all patients, $p=1$), while the integral implant functioning factor (by Mirgazizov) fell within norm in both groups.

An X-ray examination of peri-implant tissues revealed no bone resorption, which confirmed inflammatory issues only in soft tissues.

The obtained results suggest that poor oral hygiene is a real factor leading to inflammation. This fact points at the effect that the microbial factor has on the degree of inflammation developing at teeth and implants, as well as potential cross-infection from teeth to implants.

In view of the above, it is advisable to follow the oral hygiene status dynamics as well as the status of peri-implant tissues, both at the stage of implantation and implant-based prosthetics.

A comparative assessment of the clinical image presented by peri-implant mucositis in Groups I and II revealed distinctive features that can be considered as differential symptoms, whereas the entire issue requires further research.

CONCLUSION

Clinically and radiologically, peri-implant mucositis of a chronic course can be identified based on the combination of the following features: bleeding peri-implant gum; slight soreness and swelling of the peri-implant gum; lack of implant mobility; lack of peri-implant pocket; lack of peri-implant bone tissue destruction; a direct relationship between the hygiene index indicators and mucositis; lack of issues in the patient's overall condition.

Acute peri-implant mucositis was clinically diagnosed subject to a combination of the following signs: significant pain in the peri-implant gum; sharp

Table 1. *Comparative assessment of clinical and radiological signs of peri-implant mucositis (Group I and II)*

Symptom	Peri-implant mucositis; n=104			
	Chronic course (Group 1; n=83)		Acute course (Group 2; n=21)	
	abs	%	abs	%
Pain: significant	0	0	17	80.95
insignificant	57	68.67	4	19.05
none	26	31.33	0	0
Bleeding gums: seldom	0	0	0	0
often	3	3.61	0	0
always	80	96.39	21	100
Gum palpation: mildly painful	66	79.52	2	9.52
painless	5	6.02	0	0
sharpy painful	12	14.46	19	90.48
Hyperemia: insignificant	10	12.05	0	0
significant	68	81.93	3	14.29
sharp	5	6.02	18	85.71
Discharge from the peri-implant cuff: serous	28	33.7	6	28.57
purulent	0	0	15	71.43
none	55	66.3	0	0
Implant mobility: immobile	83	100	21	100
Osteoporosis: present	0	0	0	0
none	83	100	21	100
Peri-implant bone destruction: present	0	0	0	0

Table 2. *Index evaluation of oral hygiene, periodontal and peri-implant tissue status in patients with peri-implant mucositis, Groups I and II*

Peri-implant mucositis	Green-Vermillion index (OHI-S)		Loe- Silness gum index		Muhlemann index	
	Abs.	Assessment	Abs.	Assessment	Abs.	Assessment
Chronic course (Group 1; n=83)	2.5±0.21	satisf.	1.3±0.11	Mild degree	1.8±0.17	Medium inflammation
Acute course (Group 2; n=21)	3.3±0.26	poor	2.4±0.19	Medium inflammation	2.2±0.30	Severe inflammation

Table 3. *Index assessments of periodontal and peri-implant area tissues in patients with peri-implant mucositis, Groups I and II*

Peri-implant mucositis	Russel periodontal index		Tooth and implant mobility Miller-Fleszar		Integral implant functioning factor (by Mirgazizov)	
	Abs.	Assessment	Abs.	Assessment	Abs.	Assessment
Chronic course (Group 1; n=83)	1.2±0.08	Mild degree	0	Stable	1.0	Norm
Acute course (Group 2; n=21)	1.6±0.11	Medium degree	0	Stable	1.0	Norm

soreness, hyperemia and swelling of the peri-implant cuff; serous and purulent discharge; peri-implant *false pocket*; constant and significant bleeding of the peri-implant gum; a direct relationship between the hygiene index and mucositis, as well as lack of destruction in the peri-implant bone tissue.

A combination of these factors is the issue behind a fairly frequent inflammation in the soft peri-implant tissues, which, in turn, can result in a disintegrated implant.

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CHARACTERISTICS OF CYTOKINE PROFILE OF ORAL FLUID IN PATIENTS WITH CHRONIC SIMPLE MARGINAL GINGIVITIS

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ABSTRACT — Biomarkers of inflammation can be used as indicators for monitoring the treatment of periodontal diseases, as well as for finding the safest and most effective drugs with different mechanisms of action. The concentration of biomarkers in saliva was determined by enzyme immunoassay and chemiluminescent analysis. The obtained results reflect the degree of activity of inflammatory processes in periodontal tissues that occur in gingivitis and reveal an imbalance in the processes of free radical oxidation and antioxidant protection of the oral cavity.

KEYWORDS — biomarkers of inflammation, periodontal diseases, gingivitis.

INTRODUCTION

Nowadays, periodontal diseases contribute to the highest dental morbidity among the population of the Russian Federation, causing early tooth loss and negatively affecting the quality of human life [1,6].

The nonspecific mechanism of anti-bacterial protection in oral cavity is represented by several components. These include mechanical factors (barrier function of mucous membranes), microbiological component (role of normal microflora), chemical (humoral) and cellular immunological factors of the oral fluid [2]. In inflammatory periodontal diseases (IPD), the number of phagocytes is increased as a result of decreased apoptosis intensity. Activated neutrophils secrete a number of cytokines, which, in turn, prolong cellular response to the pathogen. In IPD, the imbalance between pro- and anti-inflammatory cytokines develops. Pro-inflammatory cytokines represent the central mediators in the pathogenesis of IPD. [3].

Diagnostic approaches based on the determination of molecular biomarkers of inflammation, alteration, immune system, antioxidant status, etc. have become widespread, allowing to predict the outcome of disease and effectiveness of treatment [5, 12, 17, 30]. This are primarily cytokines, defensins, cathelicidins, components of the complement system, immunoglobulins, growth factors, markers of oxidative stress [8, 9, 11, 15, 23].

Recent fundamental studies convincingly demonstrate that these biologically active compounds have a wide spectrum of action and play a decisive role in the immunopathogenesis of many diseases, including periodontal disease [4, 10]. It is known that depending on the set of secreted cytokines, transcription factors and signaling pathways, effector CD4 + T-helper lymphocytes are subdivided into Th1, Th2, Th3, and Th17 subpopulations.

Cytokines produced by Th1, in particular IFN-γ, IL-2, TNF-α, control cellular defense mechanisms through macrophages, providing a delayed-type hypersensitivity reaction and activation of cytotoxic T-lymphocytes (CD8+). The result of the action of mediators synthesized by Th2 (IL-4, -5, -6, -10) is the activation of B-lymphocytes, followed by their differentiation into plasma cells and the formation of antibody synthesis [22, 24].

Defensins act as one of the main triggers for the induction of cytokine synthesis and are involved in the gene expression regulation of most cytokine and chemokine receptors [21]. Stimulation of CD4+ T-cells with defensins increases the production of IFN-γ, IL-2, IL-6, IL-10, IL-8. HNPs stimulate monocyte production of TNF-γ and IL-1β. Defensins are also involved in the initiation of humoral response against microbial antigens, initiate the production of ovalbumin-specific IgG [26, 29].

Another important protective peptide, LL-37, is also a product of neutrophils and, to a lesser extent, epithelial gingival cells. LL-37 belongs to a group of major mammalian antimicrobial proteins called cathelicidins. The origin of the name is associated with the presence of 37 amino acids and two leucine residues at the N-terminus of the molecule [13]. The study of antimicrobial activity of LL-37 against oral bacteria showed specificity for P. gingivalis, A. actinomycetemcomitans, Streptococcus gordonii, Prevotella intermedia, Fusobacterium nucleatum and Streptococcus sanguinis, with the greatest activity noted against A. actinomycetemcomitans and Capnocytop. [19, 20].

The level of cathelicidins is subject to significant fluctuations in various pathological conditions. Thus, the concentration of LL-37 in the gingival fluid is significantly increased in patients with chronic periodontitis, but this was uncommon for gingivitis [18].

The complement system also plays a significant role in the pathogenesis of periodontal diseases [14, 15]. It was found that inhibition of complement cascade at the level of its main component C3 by local administration of the oligopeptide Cp40/AMY-101 provides a therapeutic effect, protecting primates from induced or natural periodontitis [15].

The high risk of periodontal pathology is also evidenced by the presence of signs of oxidative stress, and this phenomenon is considered as a criterion for the severity of the disease course, which can be used for timely prescription and/or adjustment of treatment [31].

The most commonly measured markers of oxidative stress are malondialdehyde and thiobarbituric acid-reactive substances (TBARS), indicating oxidative damage to lipids, especially lipid membranes. These analytes are widely used due to their low labor intensity and speed of execution, for rapid assessment of oxidative status and activity of inflammatory process in clinical medicine, including dentistry [7].

Thus, the imbalance of the pro- and antioxidant systems of plasma, saliva, and gingival fluid is an integral diagnostic sign of various clinical forms of gingivitis/periodontitis, which predetermines the need to analyze the redox state of these biological media in inflammatory processes of oral cavity. Current data do not support the use of any one universal indicator of oxidative stress. Obviously, a minimum set of markers is required, including the assessment of both oxidative damage and antioxidant status.

Particular attention is focused on the central role of oxidative stress in the pathogenesis and progression of chronic periodontal inflammation and the need to correct the redox imbalance in this area using antioxidants of various nature [27, 28].

Molecular markers of damage and adaptation have great potential for new diagnostic, preventive and therapeutic strategies in dentistry, and a deeper understanding of the mechanisms of their regulation as a component of the body's compensatory-adaptive systems is of great scientific and practical importance [16, 25]. The aim of the research is to analyze the level of biomarkers of inflammation and degree of oxidative stress in patients with chronic simple marginal gingivitis.

MATERIALS AND METHODS

All patients were divided into 2 groups — control group (n = 35) and a group of patients with chronic simple marginal gingivitis (CSMG) (n = 45). Oral fluid intake was carried out in the morning within the period from 8:00 to 10:00 a.m., on an empty stomach according to the standard method. Before taking the oral fluid, three times rinsing the mouth with saline sodium chloride solution (0.9% NaCl) was carried out. Samples of the oral fluid were collected in disposable sterile 1.5-ml Eppendorf. Delivery of biomaterial to the laboratory was carried out within an hour at a temperature of 20° to 37° C. In the summer, due to the impossibility of rapid delivery, the material was frozen, and the samples were stored in low-temperature refrigerator at –20° C for no more than 6 months.

The content of the analytes was determined by biochemical and immunological methods using standard test systems in accordance with the manufacturer's instructions. The concentration of interleukins was determined with test systems "Bender MedSystems" (Austria) on an enzyme immunoassay analyzer "Anthos 2020". The assessment of the state of free radical processes and antioxidant activity in the oral cavity was also carried out using chemiluminescent analysis with a portable domestic chemiluminometer HL-003.

RESULTS AND DISCUSSION

We studied the indicators of cytokine status, oxidative stress, activity of the complement system and the level of some antimicrobial peptides in oral fluid in patients with CSMG and patients of the control group.

Table 1 shows the results of a study of the level of some components of complement system in the oral fluid of patients with CSMG and patients of the control group.

The obtained data shows that there is a significant increase by almost 68% in concentration of C3a in oral fluid of patients with chronic simple marginal gingivitis as compared to parameters of healthy individuals — an increase in the level of C3a as indicator of hyperstimulation of complement system indicates the activation of hydrolysis of its precursor C3 component. The increase in C3a has an important independent significance in the development of gingivitis, since it is able to initiate chemotaxis of leukocytes to the focus of inflammation and release of histamine from mast cells and platelets.

The level of various antimicrobial peptides, in particular, defensins and cathelicidins, is considered as a reliable indicator of the activity of inflammatory phenomena.

As it is shown in the Table 2, the concentration of α-defensins in patients with CSMG exceeded those in the control group ($p \leq 0.05$).

Table 1. *Content of components of the complement system in the oral fluid (M±m)*

	Control group		Patients with CSMG	
	C1 inhibitor, μg/ml	C3a, ng/ml	C1 inhibitor, μg/ml	C3a, ng/ml
Indicator	34,72±3,13	36,4±7,52	13,23±3,05*	61,16±8,33*

Note: ** statistical significance of differences with the control group*

Table 2. *The content of some antimicrobial peptides in the oral fluid in patients of the control group and with CSMG (M±m)*

	Control group		Patients with CSMG	
Indicator	α-defensin, pg/ml	LL-37, ng/ml	α-defensin, pg/ml	LL-37, ng/ml
	0,82±0,09	28,4±3,51	3,15±0,41*	55,6±7,8*

Note: ** statistical significance of differences with the control group*

The analysis of the concentration of some cytokines in our patients is of particular interest (Table 3). This table also shows the analysis of matrix metalloproteinase-8 (MMP-8) activity since there is data on the high sensitivity and specificity of the combination of the determination of this enzyme together with IL-6 in the diagnosis of CSMG.

Table 3. *The concentration of some cytokines and MMP-8 in the oral fluid in patients of the control group and with CSMG (M±m)*

Group	IL-1β, pg/ml	IL-6, pg/ml	IL-33, pg/ml	ФНОα pg/ml	MMP-8, ng/ml
Control group	14,6±2,6	3,7±0,6	455,4±37,6	16,5±1,9	91,7±14,2
Group of patients with CSMG	97,3±11,4*	20,8±3,7*	692,9±48,2*	28,5±3,3*	334,1±21,6*

Note: ** statistical significance of differences with the control group*

The concentrations of all studied parameters in patients of the main group were significantly higher ($p \leq 0.05$) compared with the group of healthy patients, however, the degree of increase in their levels differed markedly.

Thus, in our work, we confirmed the results of other authors showing high diagnostic value of the combination of biomarkers IL-1β, IL-6, and MMP-8 for verifying periodontal pathology.

There is a promising approach based on the determination of combination of IL-6 and other pro-inflammatory proteins of macrophages in the oral fluid, which makes it possible to perform differentiative diagnosis of gingivitis with a sensitivity of more than 80% and a specificity of more than 70%.

The next stage of our work was devoted to the study of redox status parameters of the oral fluid in patients with CSMG and the control group. The imbalance between generation of reactive oxygen species (ROS) and antioxidant activity of the oral cavity is the main prerequisite for the development of oxidative stress, which is the leading pathogenetic factor of periodontal diseases.

The degree of oxidative stress activity was analyzed according to the level of TBA-reactive products (TBA-RP), and the antioxidant status of the oral fluid was assessed by determining total antioxidant activity (TBA). The parameters of the redox status of the oral fluid in examined patients are shown in Table 4.

The obtained data indicates that in chronic marginal gingivitis, intermediate products of lipid oxidative modification such as TBA-RP accumulate in the oral fluid with increase up to 220% as compared to the control level in patients of the main group.

The total antioxidant activity of the oral fluid in patients with gingivitis was reduced by almost one third and accounted to 69% ($p < 0.02$) from the parameters in the control group.

The obtained results reflect the degree of activity of inflammatory processes in periodontal tissues that occur during gingivitis and are accompanied with induction of lipid peroxidation.

At the next stage of our research, the state of *lipid peroxidation — antioxidant protection* in the oral fluid of patients with CSMG was studied using chemiluminescence analysis in vitro model systems generating ROS (Table 5).

CONCLUSION

Taken together, obtained results indicate the imbalance between processes of free radical oxidation and antioxidant defense in the oral cavity accompanied with the intensification of oxidative stress during CSMG.

Accordingly, such biomarkers as components of compliment system, α-defensin, LL-37, MMP-8, interleukins and TNF-α could be used as informative indicators for controlling the treatment of periodontal diseases, as well as for searching for the safest and most effective drugs with different mechanisms of action.

To conclude, a large group of biologically active compounds of protein origin is directly or indirectly involved in the realization of

Table 4. *Indicators of free radical homeostasis of the oral fluid and patients of the control group and with CSMG (M±m)*

Group	TBA-RP, nM/mg protein	Total antioxidant activity (AAA), nM/ml
Control group	4,19±0,58	2,14±0,19
Group of patients with CSMG	9,23±0,87*	1,45±0,12*

Note: ** statistical significance of differences with the control group*

Table 5. *The level of chemiluminescence (in conv. units) of the oral fluid in patients of the control group and with CSMG (M±m)*

Group	Emission of light	Spontaneous luminosity
Control group	3,83±0,41	1,49±0,15
Group of patients with CSMG	7,15±0,69*	4,43±0,39*

Note: ** statistical significance of differences with the control group*

pathogenic mechanisms in CSMG, including substances with different diagnostic and prognostic potential.

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BIOLOGICAL MARKERS OF MMP-8 (MATRIX METALLOPROTEINASE 8) AND TIMP-1 (TISSUE INHIBITOR OF METALLOPROTEINASES 1) IN THE ORAL FLUID IN VARIOUS FORMS OF LICHEN PLANUS AND THEIR ROLE IN ASSESSING THE CLINICAL COURSE OF THE DISEASE

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ABSTRACT — Early detection of tumor transformation of lichen planus presents a real challenge, since squamous cell carcinoma can develop both from unchanged epithelium, and against the background of inflammatory or precancerous lesions. Another aspect of untimely diagnosis is the unfavorable long-term prognosis of lichen planus, so after complex treatment, 50% of patients have a relapse: 80% — within 2 years and 20% — within 4 years. The aim of this study was to analyze the levels of MMP-8, TIMP-1 and their ratio of MP-8/TIMP-1 in the oral fluid of patients with varied forms of lichen planus. We examined and treated 24 women (35–70 years old). 14 women with oral lichen planus of the mucosa in a typical form were assigned to Group 1; 10 patients with an exudative-hyperemic form - Group 2. Concentrations of the biological markers MMP-8 and TIMP-1 were determined in the oral fluid before and after treatment. Results of the study showed that to assess the severity of oral lichen planus it is essential to evaluate concentration ratio of biological markers MMP-8/TIMP-1 in mixed saliva. Dynamic changes in MMP-8/TIMP-1 levels should be monitored at least once in a quarter.

KEYWORDS — lichen planus, oral fluid biomarkers, MMP-8, TIMP-1.

INTRODUCTION

Lichen planus is a chronic recurrent inflammatory disease, with a prevalence of 1–2% among the adult population, more common in women [1], has both separate manifestations on the skin, mucous membranes, and combined [2]. The probability of neoplastic transformation of lichen planus is from 0.4 to 6.5%, in this regard, the World Health Organization estimates lichen planus as a precancerous condition [7]. The greatest difficulties are the early detection of tumor transformation of lichen planus, since squamous cell carcinoma can develop both from unchanged epithelium, and against the background of inflammatory or precancerous lesions. Another aspect of untimely diagnosis is the unfavorable long-term prognosis of lichen planus, so after complex treatment, 50% of patients have a relapse: 80% — within 2 years and 20% — within 4 years [9].

Currently, the pathogenetic mechanisms that initiate the development of oral mucosal cancer in patients with lichen planus [2, 4, 17, 19] are not fully investigated. However, it is recognized that endogenous factors can play an important role in the malignancy process. Matrix metalloproteinases (MMP) [18, 8] are considered to be one of these factors.

MMP are a large family of zinc-dependent endopeptidases (about 30) that are capable of destroying all components of the basement membrane and extracellular matrix (ECM). Often there is a cascade activation of several MMP at once, which increases both in inflammatory and malignant diseases [18, 20, 8]. The increased expression of MMP increases the invasive activity of tumor cells, which can penetrate into the surrounding organs and tissues, as well as affect the processes of growth, migration, apoptosis and angiogenesis.

Researchers are particularly interested in the neutrophil collagenase MMP-8, which is involved in reparative processes in ulcerative lesions of the mucous membrane. It is believed that MMP-8 is associated with pathogenetic mechanisms for maintaining long-

term chronic mucosal lesions due to the cleavage of type I collagen, while there is a significant increase in MMP-8 levels [14, 15]. A number of authors believe that the increased collagenolytic activity of MMP-8 is associated with a decrease in the level of the tissue inhibitor of matrix metalloproteinases type 1 (TIMP-1) [9, 15].

TIMP-1 plays an important role in maintaining the integrity of tissues, and has recently become a decisive factor in the assessment of a number of pathological conditions. The versatile effect of TIMP-1 on cellular functions is due to the duality of the structure containing both an MMP inhibitor and a cytokine activator. This feature leads to interactions with numerous cell surface proteins that initiate an exceptionally wide range of effects, which explains the diverse biological consequences of TIMP-1 expression [9, 16].

Almost all components of ECM degrade under the influence of endogenous MMP. The early development of the tumor, as well as distant metastases, may be the result of an imbalance in the MMP/TIMP ratio, which changes the cell structure [5, 8].

In the oral cavity, biomarkers are directly or indirectly released into mixed saliva, so its use as a diagnostic fluid has prognostic value in various diseases [6, 12, 13, 14, 21]. The oral fluid washes the elements of the affected oral mucosa, thereby maintaining the concentration level of the studied proteins.

According to current literature data, the development of lichen planus is due to multifactorial causes, and therefore, there are various treatment protocols and clinical recommendations that lead to temporary improvement and prolongation of the remission period [11]. The polymorphism of the course of lichen planus complicates the determination of the individual prognosis of the course of the disease. The study of biomarkers in the oral fluid allows us to evaluate the effectiveness of therapy and the duration of *light* periods.

The aim of this study was to analyze the levels of MMP-8, TIMP-1 and their ratio of MP-8/TIMP-1 in the oral fluid of patients with various forms of lichen planus.

MATERIALS AND METHODS

In 2019–2020, 24 women (35–70 years old) with a diagnosis of lichen planus of the oral mucosa were examined and treated at the Central State Medical Academy of the Department of Presidential Affairs (Moscow, Russia). Depending on the form of lichen planus, the patients were divided into 2 groups: 1) a typical form (14 patients), 2) an exudative-hyperemic form (10 patients).

The criterion for excluding patients from the study was the presence of pathologies of the oral mucosa: infectious, allergic, benign neoplasms, leukoplakia.

The control group consisted of 19 practically healthy donors aged 45 to 55 years without lesions of the oral mucosa.

The examination was carried out by standard methods: a survey, anamnesis collection, when describing the external status, special attention was paid to the state of the lymph nodes of the regional region. Examination of the oral mucosa included registration of the condition of the mucous membrane of the lips, cheeks, hard and soft palate, gums, teeth, dentition, identification of the source of permanent trauma (dystopian teeth, sharp edges of teeth, fillings, orthopedic structures, the presence of dissimilar metals), determination of the indices: CFR and oral hygiene.

They carried out professional hygiene, eliminated traumatic factors, replaced amalgam fillings and fillings with a broken edge fit, not high-quality orthopedic structures made of dissimilar metals. They gave recommendations on the diet (they excluded hot, spicy, and acidic foods). They were assigned to consult internists to determine the general treatment of lichen planus. Each patient was given an individual therapy plan aimed at the pathogenetic links of the disease and, taking into account the presence of concomitant somatic pathology, the course necessarily included the appointment of antioxidants, vitamin therapy.

Local treatment consisted of anti-inflammatory therapy with solutions of antiseptics based on chlorhexidine 0.05% in the form of oral baths with an exposure of 1 minute. We prescribed applications of oil solutions of vitamin A and E on gauze napkins for 15–20 minutes, which reduce the process of keratinization and affect the proliferation of epithelial cells. An immunocorrective drug was used to activate phagocytosis and produce immunoglobulin A. The course of therapy was 14 days, the drugs were used 3 times a day.

Clinical observations were carried out at the stage of diagnosis, after 14 days of treatment.

At the specified time, unstimulated oral fluid was collected in a graduated tube on an empty stomach in the morning at rest. The mouth was rinsed with water. Oral fluid with a volume of 5 ml was obtained by spitting in the absence of chewing movements, frozen and transported in a refrigerator bag to the laboratory of Clinical Biochemistry of the N.N. Blokhin National Research Center of Oncology of the Ministry of Health of the Russian Federation and stored at-80o before the study for 1–2 months.

The concentration of MMP-8 and TIMP-1 in oral fluid samples was determined using reagent kits for direct enzyme immunoassay "Human MMP-8 Immunoassay "(R&D Systems, USA) and" TIMP-1 ELI-

SA " (Bender Medsystems GMBH, Austria) according to the manufacturer's instructions, as described earlier [13]. The concentration of the studied proteins was expressed in nanograms (ng) per 1 ml of oral fluid.

Statistical processing of the results was carried out using the central characteristic – the median, quartiles were used to estimate the spread of indicators, and nonparametric methods of analysis were used for comparison: the Mann–Whitney test (U test) when comparing independent groups, and the Wilcoxon paired test when evaluating the dynamics of marker levels. The differences were considered statistically significant at $p<0.05$

RESULTS

Patients with a typical form of lichen planus complained of discomfort, roughness of the mucous membrane; with an exudative-hyperemic form-burning when eating irritating food, discoloration of the cheeks, tongue.

In the oral cavity with a typical course of the disease, grayish papules were recorded on the unchanged oral mucosa (Fig. 1).

The exudative-hyperemic form of lichen planus was characterized by the presence of single or multiple elements of the lesion, connecting in various patterns in the form of a grid, a ring, leaves against the background of a hyperemic mucosa (Fig. 2).

After the treatment, there was a regression of the lesion elements in the typical form of lichen planus, an unexpressed lichenoid reaction was noted (Fig. 3).

In group 2, the papules were pronounced, but they were located on the pale pink mucous membrane of the oral cavity (Fig. 4).

A comparative analysis of the medians of the concentration of TIMP-1 in the oral fluid of patients with typical and exudative-hyperemic forms of lichen planus before treatment and in the control did not reveal statistically significant differences (p=0.49). The median concentrations of TIMP-1 were almost the same, amounting to 619 and 625 ng/ml, respectively. The median TIMP-1 in the group of patients with the typical form of PL was 616; 612-637 ng / ml (Table. 1; Fig. 5).

There were statistically significant differences in the concentration of MMP-8 in the oral fluid of the general group of patients with lichen planus and in the control group (p=0.0006), the medians were 311 and 210 ng / ml, respectively (Table. 2; Fig. 6). The frequency of detection of MMP-8 levels above the upper limit of control (335 ng/ml) in the general group of patients with lichen planus was 43% (p=0.014 according to the exact Fisher criterion), that is, almost half of the examined patients with lichen planus observed an excess of MMP-8 concentrations in the oral fluid relative to healthy donors.

At the same time, the median MMP-8 in the oral fluid of patients with typical lichen planus was the lowest 303; 285–556 ng/ml, while in exudative-hyperemic patients it corresponded to 370; 310–429 ng/ml.

The excess of MMP-8 concentrations relative to the control group was recorded, which was lower in group 1 (37.5%), and in group 2 was 50%.

Against the background of treatment, the values of the MMP-8 index decreased by 2 times during the repeated study.

At the same time, the median concentration of MMP-8 in the oral fluid of patients with lichen planus in the typical form was more than 2 times higher than in the exudative-hyperemic form of lichen planus (medians 311 and 117 ng/ml, respectively), but this difference did not reach the level of statistical significance.

Statistically significant differences were also found when comparing the ratio of MMP-8 and TIMP-1 levels in the oral fluid of patients with lichen planus and in the control (p=0.006). It should be noted that in 43% of patients with this pathology of the oral mucosa, the ratio of MMP-8/TIMP-1 was exceeded compared to the maximum value of this ratio in the control (p=0.0016). At the same time, the median ratio of MP-8/TIMP-1 in the group with a typical form of PL reached 0.55 and was significantly lower than in the group with exudative-hyperemic — 1.06 (Table 3; Pic. 7).

When repeated studies were performed in patients with lichen planus, the concentration of MMP-8 decreased by 1.3–6.2 (median 3.3; p=0.028), and the concentration of TIMP-1 — by 1.1–3.1 (median 1.57; p=0.028). There was a statistically significant decrease in the ratio of MP-8/TIMP-1 (Fig. 7; p=0.043), in three patients this indicator increased.

DISCUSSION

The study demonstrated an increase in the concentration of MMP-8 in the oral fluid of patients with typical and exudative-hyperemic forms of lichen planus at the stage of diagnosis of the disease compared to healthy donors, and the increase in this marker was most pronounced in patients with exudative-hyperemic forms of lichen planus.

The levels of TIMP-1 in patients with the studied forms of lichen planus did not differ from those in the control group.

At the same time, the ratio of MMP-8 and TIMP-1 concentrations was significantly higher than in the control group. It can be assumed that the low level of TIMP-1 in patients with lichen planus does not suppress the collagenolytic activity of MMP-8,

Fig. 1. Patient P., 53 years old. The clinical picture of the typical form of lichen planus before treatment. Papules are located on the mucous membrane of the cheeks on the right and left along the line of closing of the teeth.

Fig. 2. Patient H., 47 years old. Clinical picture of exudative-hyperemic form of lichen planus before treatment. Elements of the lesion on the hyperemic mucous membrane of the cheeks on the left and right sides.

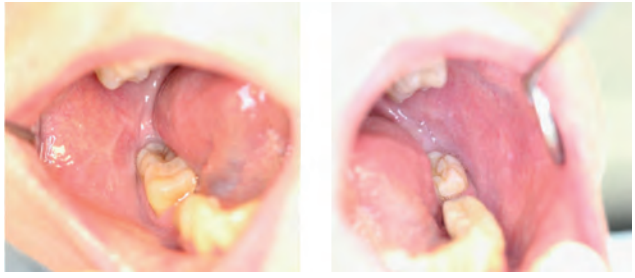

Fig. 3. Typical form of lichen planus after treatment.

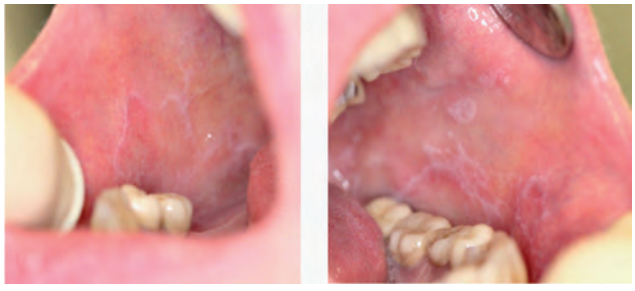

Fig. 4. Exudative-hyperemic form of lichen planus after the treatment.

***Table 1.** The content of TIMP-1 in the oral fluid of patients with lichen planus and in the control before treatment*

Group	N	TIMP-1, ng/ml			P
		Limits	Median	Quartiles	
Control	19	91,1-1825	625	610-1128	>0,05
Lichen planus (general group)	24	141-1659	619	610-776	

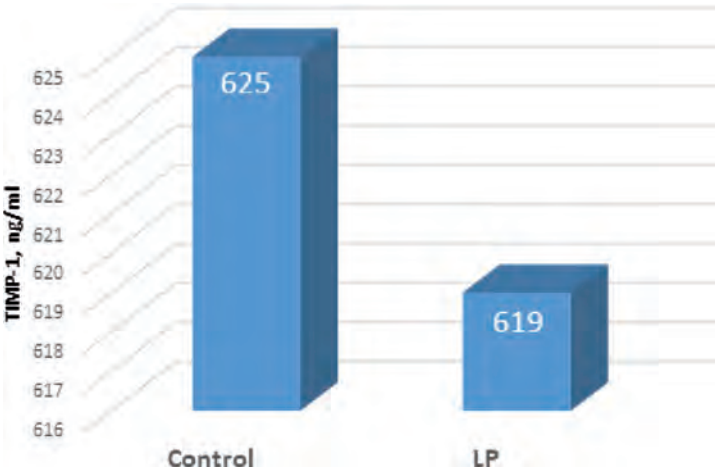

Fig. 5. Concentrations of TIMP-1 in the oral fluid of patients with lichen planus and in the control group

***Table 2.** The content of MMP-8 in the saliva of patients with lichen planus and in the control*

Group	N	MMP-8, ng / ml			P
		Limits	Median	Quartiles	
Control	19	28,7-335	210	115-262	0,0006
Lichen planus (general group)	24	144-3294	311	284-742	

which causes the severity of clinical manifestations, especially in group 2.

After the treatment, 75% of patients showed a statistically significant decrease in the studied parameters (MMP-8, TIMP-1, MMP-8/TIMP-1), which correlates with the elimination of the inflammatory reaction of the oral mucosa.

A decrease in the levels of MMP-8, TIMP-1 and their ratio after the therapy indicates a favorable course of the disease due to the inhibition of the functional activity of neutrophil collagenase

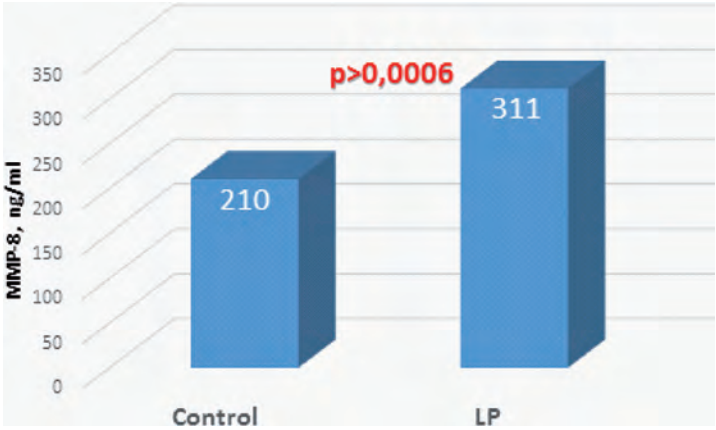

Fig. 6. Concentrations of MMP-8 in the oral fluid of patients with lichen planus and in the control.

Table 3. The ratio of MMP-8/TIMP-1 in the oral fluid of patients with lichen planus and in the control

Group	N	MMP-8/ TIMP-1 ratio			P
		Limits	Median	Quartiles	
Control	19	0,03-0,85	0,25	0,17-0,42	0,006
Lichen planus (general group)	24	0,15-5,21	0,55	0,34-1,78	

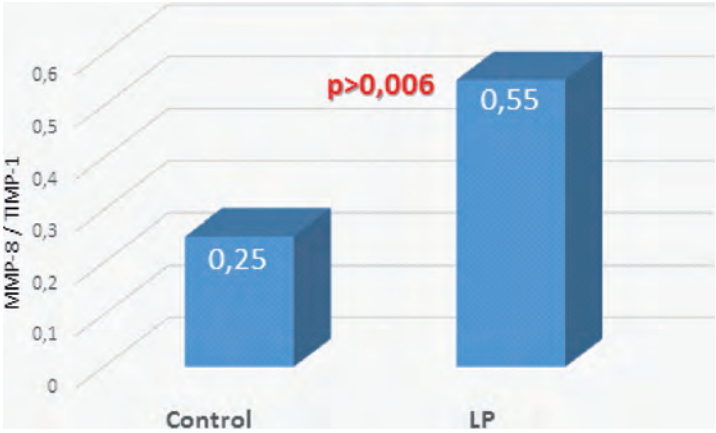

Fig. 7. The ratio of MMP-8/TIMP-1 in the oral fluid of patients with lichen planus and in the control.

MMP-8, which contributes to the preservation of the integrity of the mucosal tissues and prevents the development of the tumor process.

By the ratio of MMP-8/TIMP-1, we can judge the prognosis of the disease: the duration of remission and possible relapses. In the group with exudative-hyperemic form, the MMP-8/TIMP-1 imbalance was 2 times higher than in the typical course, this fact explains the appearance of a relapse of the disease after 3 months in the form of the addition of a pain factor and hyperemia of the oral mucosa along the periphery of the lesion elements.

CONCLUSION

To determine the prognosis of the course of the disease in patients with various forms of lichen planus of the oral mucosa, it is necessary to evaluate the ratio of the concentration of biological markers MMP-8/TIMP-1 in mixed saliva.

Dynamic monitoring of changes in the oral fluid level of MMP-8/TIMP-1 should be carried out at least once a quarter to assess the possible risks of relapse.

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THE ROLE OF PSYCHOEMOTIONAL STRESS IN THE DEVELOPMENT OF INFLAMMATORY POST-PROSTHETIC COMPLICATIONS

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ABSTRACT — Relevance: Before starting the treatment, the patient's medical history should be carefully studied. Special attention should be paid to the presence/absence of diseases of the cardiovascular system, kidney disease, endocrine pathology, and the psychological state of the body. These diseases can directly affect the dental status and worsen the clinical picture. Psychoemotional stress and inflammatory post-prosthetic complications are reciprocal risk factors. Psychophysiological disorders can lead to the development of dental complications in the oral cavity and vice versa, mucositis can cause a stress reaction in a person. This assumption served as the purpose of this study. Materials and methods: During implant treatment, the analysis of the psychophysiological state in patients with inflammatory post-prosthetic complications was carried out according to 3 parameters (the results of the questionnaire, based on heart rate variability and using software), parallel to the assessment of the dental status in 30 patients. Stress and mucositis should be treated simultaneously, with sedation support where necessary. Outcomes: All patients showed a high level of stress. During the dental examination-the presence of inflammation of the parotid tissue was detectes. Conclusion: Psychoemotional stress can be one of the initiating factors in the development of inflammatory complications during dental implantation. The main task of a dentist is, first of all, to identify the early stages of the development of the disease, and secondly, to provide not only symptomatic, but also pathogenetic treatment, taking into account the factors that facilitate the development of this pathology.

KEYWORDS — stress, mucositis, psychoemotional stress, diagnosis, treatment.

INTRODUCTION

Changes are observed in the oral cavity, the severity of which depends on the severity of the course, the age of the patient and the duration of the course of the general somatic pathology [12]. The most characteristic changes in the oral cavity are xerostomia, catarrhal stomatitis and glossitis, fungal stomatitis, mycotic congestion, paresthesia of the oral mucosa, trophic disorders, lichen planus, gingivitis, periodontitis, mucositis [9]. At the same time, a two-way relationship is monitored [8], as inflammatory diseases in the oral cavity can contribute to the occurrence and exacerbation of chronic diseases of the body, and general somatic pathology can cause changes in the oral cavity [1–6]

Psychoemotional stress is also no exception in the development of inflammatory complications in the oral cavity [10]. It is known that stress is an adaptive syndrome, which is a set of non-specific reactions of the body that mobilize all the resources for a specific restructuring of its various systems. The sympathetic nervous system works as a stress-implementing system, that is, it mobilizes the body's defenses in response to a damaging factor, which, in turn, leads to neuropathologies with prolonged exposure [7]. The humoral component also refers to the stress-implementing system due to the work of hormones of the cortical and cerebral layers of the adrenal glands, as well as thyroid hormones, which leads to endocrine pathology. Early diagnosis of concomitant pathology and timely treatment [11] are one of the key factors for the success of dental treatment of inflammatory diseases of the oral cavity.

The aim of the study is to analyze the influence of predisposing factors on the development of inflammatory periodontal diseases.

MATERIALS AND METHODS

The emotional and personal characteristics of the patients were evaluated using the following methods:

1. Software based on photoplattismography.
2. Questionnaire "PSM-25 scale of psychological stress".
3. LDF-metric data for calculating the centralization index-IC.

To calculate the stress level using software based on photoplattismography, formulas for estimating heart rate variability were used. The actual heart rate of the patient was compared with the normal value of this indicator in healthy people of the corresponding gender and age. The following gradation of the stress level was used: low, medium, high.

Based on the sum of the points received when answering the questions of this questionnaire, we

determined the level of stress of patients. The following gradation of the final result was used: less than 99 conv. units — low level of stress; 100–125 conv. units — average level of stress; more than 125 conv. units — high level of stress.

The assessment of heart rate variability was carried out using the software and hardware complex "Poly-Spectrum" (version 1.0.1.3; LLC "Neurosoft", Ivanovo).

The analysis of the dental status was carried out on the basis of a clinical examination and additional methods of examination. The assessment was performed in 1788 people aged 18–44 years (WHO average age) who sought dental care. The plaque index — PCR, the Schiller-Pisarev test, the Svrakov iodine number, the VOR bleeding index, the periodontal screening — PSR, and the implant stability coefficient (CI) were evaluated. To assess the radiological characteristics, three-dimensional computed tomography and targeted dental radiography were used. The study was conducted on a Hyperion X9 dental digital tomograph (MyRay, France). The shooting mode was 60–75 kW, 7–10 mA, the duration of the system movement was 10–13 seconds. The condition of the existing teeth, periapical tissues, jaw bone tissue, resorption, and osteoporosis of the peri-graft bone tissue were evaluated. Targeted dental radiography was performed on an Evolution X-ray machine (Italy). Shooting mode: 70 kW, 4 mA, the time was adjusted depending on the clinical situation. Sighting images allow you to get the most objective data on the condition of the bone adjacent to the implants, characterized by a minimum of distortion.

The analysis and statistical processing of the research results was carried out by the method of mathematical statistics using a personal computer and the program "Microsoft Excel" to the software operating system MS Windows XP /Microsoft Corp., USA/ in accordance with the generally accepted methods of medical statistics, as well as the statistical software package Stat Soft Statistica v6.0. The data analysis was carried out using descriptive statistics. For each parameter, the following values were calculated: the arithmetic mean values (M), its average error (±m). The significance of the differences between the groups (p) was evaluated by the Student's criterion (t). The differences were considered statistically significant at $p<0.05$ and at $p<0.01$; $t \geq 2$.

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

RESULTS

Out of the total number of patients, 214 patients had post-prosthetic complications with dental implants. At the same time, 185 patients had disorders of the psychophysiological state of the body, characterized by tension, which probably occur under the influence of a strong emotional load. Also, the patients had a feeling of anxiety, anxiety, loss of strength, and the manifestation of negative emotions. According to the survey data, a high level of stress was revealed – 154.8±12.4 Iu, standard units. High levels of stress were also shown by data obtained using software based on photoplattismography. The value of the centralization index (the ratio (LF+VLF)/HF, reflecting the degree of predominance of the activity of the central circuit of regulation over the autonomous one) before the start of therapy was 5.8 standard units. Thus, for the group of patients, the conclusions about the level of stress obtained with the help of special medical equipment were fully consistent with the conclusions made on the basis of self-assessment and data from household electronic devices.

During the survey and dental examination, patients complained of pain, swelling, bleeding of the parotid region, and discomfort during meals. In rare cases, loss of appetite, weight loss, increased body temperature for a long period, inflammation of the submandibular lymph nodes. Objectively, the oral mucosa is swollen, hyperemic, with signs of bleeding during probing. Radiologically, there are no changes, there is no bone loss in this pathology. On objective examination, all patients had poor oral hygiene, the simplified plaque index PCR was 72.7±1.79%, while the Schiller-Pisarev test was positive, and an intense inflammatory process was observed – the Svrakov iodine number was 3.7±0.3 points (Fig. 1).

The VOR bleeding index is 57.0±3.5 points. The PSR (periodontal screening) score was 1.7±0.2, which indicates the need to remove plaque and soft residues, in some cases tartar. This indicator confirms the diagnosis of mucositis. The implant stability coefficient (CI) was 62.3±2.0, which characterizes a relatively high stabilization of the implant (Fig. 2).

In 45 patients, edema of the perimplant tissue was observed in the oral cavity, the gum mucosa around the implant is bright red, and bleeds when probing. In some cases (8 people), there was suppuration with the formation of a fistula. The gum mucosa is loose, exfoliates from the implant by an average of 1.0–1.5 mm with the formation of a periodontal pocket (Fig. 3).

At the same time, patients complained of pain, especially when chewing, pressing, and even with a simple touch of the tongue. At the same time, the implant is mobile, in some cases with displacement. The implant stability coefficient was 35.2±1.8, which indicates a low stability of the artificial tooth. The depth of the pathological peri-implant pocket was 4.7±0.3

Fig. 1. Conducting the Shiller-Pisarev test

Fig. 2. Determination of the implant stability coefficient in the oral cavity

Fig. 3. Clinical picture of peri-implantitis

mm. The data of the X-ray examination corresponded to the clinical picture. The study of sighting images and orthopantomogram data showed the presence of horizontal and vertical bone resorption of the alveolar process around the implant from 0.25 of its length to complete destruction. Foci of osteoporosis and osteosclerosis with a decrease in bone mineral density were rarely observed. The clinical and radiological picture corresponded to the diagnosis of *peri-implantitis*. This disease is irreversible, often ends with the rejection of the implant and removal.

When interviewing and filling out questionnaires about the identification and presence of stress in patients, no one was interested in this. The treatment is based only on local anti-inflammatory therapy.

CONCLUSION

All patients with post-prosthetic complications with dental implants showed a high level of stress, which is confirmed by the conclusions made on the *PSM-25 Psychological Stress Scale* and the mobile application. The presence of psychoemotional stress can be a triggering factor for the development of inflammatory complications after dental implantation. The main task of a dentist is, first of all, to identify the early stages of the development of the disease, and secondly, to provide not only symptomatic, but also pathogenetic treatment, taking into account the factors that provoke the development of this pathology, while it is necessary to reduce the time of exacerbation, increase the periods of remission. The solution of these problems is facilitated by conducting a thorough diagnosis, drawing up a treatment plan and preventive programs at a dental appointment.

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