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EDITORIAL

Prof. Dmitry Domenyuk

Executive Editor,
Archiv EuroMedica

Monitoring Blood Rheology in Diabetes Mellitus

Dear clinicians, researchers, colleagues and friends!

Diabetes mellitus is recognized by World Health Organization as non-infection epidemic of XXI century. It poses a global medical and social threat to individual health and society and occupies a leading place on growth of incidence, risk of complications, the level of disability and premature mortality. The medical, social and economic problems caused by diabetes and its complications are urging to adopt active measures aimed at mitigating its burden.

According to The International Diabetes Federation, 463 million people on the globe affected by diabetes (in Russia — 8,3 million people), almost 63% of them are of working age, however during the period 1980–2018 the total number of people with diabetes rose by 4 times.

Type 2 diabetes accounting for 85–95 % of overall prevalence of diabetes has a blurred, hidden duration. It is diagnosed occasionally, often with late diabetic complications in more than third of the patients. The important role in pathogenesis of diabetic complications is played not only by impairments in microcirculation but by deterioration of blood rheology.

Cerebrovascular and ischemic heart disease refers to the key factors of the increasing prevalence and mortality of diabetes. In the total structure of mortality among patients with type 2 diabetes, cerebral circulatory disorders are estimated as 12,17%, whereas myocardial infarction — 4,37%. It is worth mentioning that the incidence of cerebrovascular disease is estimated as 25,9 cases and its prevalence in patients with type 2 diabetes as — 428,8 cases per 10 000 adults.

Current advances in angiocardiology and neurology enable to determine a mechanism for the development of cerebral ischemia. Moreover, sometimes, the leading role in the genesis of stroke is seen in transformation of blood biochemistry, disturbance of homeostasis and fibrinolysis as well as dysfunction of the endothelia. Specialists note that even temporary hyperglycemia induces long-term epigenetic changes, persistent hyperproduction of reactive oxygen species and reduced activity of antioxidant ferments. Such changes lead to endothelial dysfunction, apoptosis, chronic inflammation in vascular wall and facilitate the development of atherosclerosis as a major vascular process and an underlying mechanism of the cerebral disease.

Subacute cerebrovascular disease in type 2 diabetes occurs due to reduction of the necessary perfusion of the brain and is accompanied with progressive disorders affecting gait and pelvic organs; emotional problems and cognitive decline. Modern researches have accumulated the evidence that type 2 diabetes is associated with the development of cognitive deficits and dementia.

Our knowledge on diabetes is constantly expanding. Novel advances are incorporated into medical practice in the shortest time. In the arsenal of physicians there are effective and safe medications, devices for self-control that provide better detection, therapy and control of this chronic condition.

In the section *Clinical Laboratory Diagnosis* you will find a paper where rheological properties of peripheral blood in patients with type 2 diabetes of different duration are discussed. Using HbA1c monitoring as the basic diagnostic and prognostic criterion for the course and outcome of cerebrovascular disease in patients with endocrinopathy allows to choose an adequate pathogenetic and personified therapy and to improve the quality of life and outcomes in this popu-lation.

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NEUROPSYCHIATRIC DISORDERS AND PARENTAL STRESS DURING THE COVID-19 PANDEMIC: AN ITALIAN RETROSPECTIVE LONGITUDINAL STUDY

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ABSTRACT — Aim: The objective of our study is to evaluate the impact that the COVID-19 emergency and the related measures adopted have had on the family management of minors with neuropsychiatric disorders. Another objective of this study is to carry out a first longitudinal evaluation of this impact on parental stress, comparing the data collected before the pandemic with those collected during the lockdown.

Methods: This is an observational study that involved 271 families of patients already treated at the Child Neuropsychiatry Unit of the Salerno University Hospital between 2 and 23 years (112 with Autism Spectrum Disorder, 86 with epilepsy and 73 with other disorders of neurodevelopment). All participants were given an ad hoc telephone interview and a standardized questionnaire (PSI - Parenting Stress Index-Short Form). The telephone interview showed that a significant percentage of parents reported an increase in their child's daily management difficulties during the lock-down and emotional / behavioral problems, in particular the externalizing disorder. Comparison of the mean scores of the PSI-SF questionnaires completed before and during the lockdown showed a statistically significant increase in scores in the Total Stress scale and in the Parental Distress subscales.

Results & Conclusion: The results of our study suggest that the confinement measures and changes in daily routine imposed by quarantine negatively affected the behavioral and emotional dimensions of both children and parents causing a significant increase in parental stress, which is mainly related to feelings of inadequacy in relation to their role in such a delicate situation, and concern for the future.

KEYWORDS — Covid-19; children; autism spectrum disorder; epilepsy; neurodevelopmental disorder.

INTRODUCTION

On January 7, 2020, the Chinese Center for Disease Control and Prevention, analyzing samples taken from the lower respiratory tract of pneumonia patients of unknown etiology, residing in the city of Wuhan, identified a new coronavirus called SARS-Cov-2. The infection caused by this virus has been called, by WHO, COVID-19 (Park, 2020). The rapid worldwide spread of the virus prompted the World Health Organization to declare the sixth public health emergency of COVID-19 of international concern (Public Health Emergency of International Concern — PHEIC), as established by the International Health Regulations (IHR, 2005, cited in Lai et al., 2020) and declare the international focus of the SARS-Cov-2 coronavirus infection a pandemic, which has caused more than 118,000 confirmed cases in 114 countries and 4,291 deaths (opening provisions of the WHO Director-General at COVID-19 media briefing: 11 March 2020). Until a vaccine and a cure are widely available, other mitigation measures are being taken to help slow the spread of the virus (Qualls et al., 2017).

Italy in February 2020 became the epicenter of the COVID-19 disease in Europe, for which the Italian government implemented more restrictive measures to avoid a rapid spread of the infection, starting the lockdown on March 9, 2020. Some of the measures adopted by the Government were: obligation to stay in one's own homes, movement possible only in situations of need and with self-certification; mandatory use of the mask outside the home; distance learning start (DAD); closure of all recreational and sports activities (cinemas, theaters, museums, gyms, etc.). These restrictive measures have weighed economically and psychologically on the population (Bloshchynskyi et al., 2021), due to the fear of contagion and the limitation of personal freedom (Tamer Bakar & Akyürek, 2020) and the drastic reduction of interpersonal relationships (Luca et al., 2020a; Grigoras & Ciubara 2021). These profound changes have led to an understanding of how children and adolescents with neuropsychiatric disorders and their families have lived through this period (Luca et al. 2020b, Baroiu et al., 2021).

The purpose of our study was to evaluate the impact that the COVID-19 emergency and the related measures adopted (closure of schools, closure of rehabilitation centers, traffic ban, etc.) have had on the

family management of children. with neuropsychiatric disorders. Another goal of our study is also to carry out a first longitudinal evaluation of this impact on parental stress, comparing the data collected before the pandemic with the data collected during the lock-down.

METHODS

Participants

The study was conducted on 271 families of patients already under treatment at the Child Neuropsychiatry Unit of the University Hospital of Salerno, aged between 2 and 23 years, of which 41.33% diagnosed with Autism (group A), 31.73% diagnosed with Epilepsy (group B), and the remaining 26.94% had a different diagnostic picture (specific learning disabilities, intellectual disabilities, attention deficit hyperactivity disorder ADHD) grouped into a only category of other neurodevelopmental disorders. The number of families in the study initially was 277, but 6 of these chose not to participate. There were no exclusion criteria except for poor parental compliance.

Assessment

All participants were given an ad-hoc phone interview and a standardized questionnaire (PSI - Parenting Stress Index-Short Form) (Reitman, Currier & Stickle, 2002) to assess stress levels within the parent-child relationship.

Telephone interview –the telephone interview is based on 16 total questions created ad hoc to collect general demographic information (e.g. age of parents and their level of education), but above all to investigate how changes in life habits related to lockdown have affected psycho-well-being -physical physique of their children (for example, type of diet, sleep rhythms, increase in problem behaviors, etc.), given the problems related to the pre-existing clinical picture, and the concerns related to the spread of COVID-19 (eg fear of contagion).

The interview includes open and closed questions, and others whose answer is based on the choice of a value on a scale from 1 to 10, where 1 corresponds to the non-occurrence of the situation under investigation and 10 to a high frequency or intensity with which this situation occurs.

Parenting Stress Index-Short Form (PSI) — the Parenting Stress Index-Short Form (PSI-SF) is a self-report questionnaire filled in by parents of children and adolescents in order to investigate their level of stress The questionnaire are divided into three subscales: the Parental Distress (PD) subscale defines the level of stress that the parent is experiencing in his specific role due to personal factors; the Parent–Child Difficult Interaction (P-CDI) sub-scale concerns the relationship with the child perceived by the parent as difficult; The Difficult Child (DC) sub-scale analyzes the characteristics of the child's behavior and the parent's perception of having a difficult child, focusing on the child's temperament and behaviors. In addition to the scores in these three areas, a Total Stress score is obtained from the sum of the three areas.

RESULTS

Telephone interview — from the information collected through the telephone interview it emerged that only 21.03% of parents did not find any increase in the difficulties of daily management of their child during the lock-down; On the other hand, 26.94% recorded an increase, albeit modest, 25.09% considered this increase to be quite significant, 26.94% recorded a significant increase in the difficulties of managing children. The 42.47% of parents of patients belonging to group C (other neurodevelopmental disorders) experienced a sharp increase in difficulties in the quarantine period, followed by 23.21% of parents of patients in group A (Autism Spectrum Disorder) and by 18.60% of the parents in group B (Epilepsy). Among the factors that have had the greatest impact on changes in child management, we find the obligation to stay at home (21.98%), the closure of school and distance learning (16.10%) and the change of daily routine (14.86%). In 10.22% of cases, the suspension of home and center rehabilitation therapies had a negative impact. Only 10.33% benefited from telematic rehabilitation therapies, while the remaining 89.67% no longer continued with any type of rehabilitation. In 47.49% of cases the parents reported a worsening of clinical symptoms, which in 12.18% was severe (of these, 31.04% were parents of children with autism, 46.2% with other disorders of the neurodevelopment and 22.7% with epilepsy).

The 47.61% of parents observed an increase in externalizing problems in their children: 19.19% in mild form, 16.6% in moderate form and 11.81% in severe form. The increase in internalization problems in children was noted by 20.3% of parents; in 8.12% the increase was slight, in 8.49% moderate and in 3.69% a serious deterioration. The 55.35% of parents were very worried about the situation created by the spread of the virus, in particular the fear of contagion, confirmed by 70.11% of the interviewees, followed by the fear that an aggravation of the child's pathology could occur (43.17%) and that the right assistance is not guaranteed in case of need (28.41%), 25.83% of the interviewees said they were also worried about the difficulties in the daily management of the child and 18.45% for the increase in problem behaviors.

Parenting Stress Index-Short Form (PSI) — By comparing the average scores that emerged from the PSI-SF questionnaires completed before the lockdown (Time 0) and during the lockdown (Time 1) it was possible to highlight some significant differences. In particular, during the quarantine, there was a statistically significant increase in scores on the Total Stress Scale (TS) and in the Parental Distress (PD) subscale.

There is a statistically significant positive correlation between the parental distress (PD) subscale and the age of the father ($r = 0.269$; $p = 0.002$) and mother ($r = 0.193$; $p = 0.030$). Furthermore, the Parent-Difficult Child Interaction (P-CDI) subscale was significantly correlated with the age of the child: higher ages corresponded to higher dysfunctional interaction scores ($r = 0.226$; $p = 0.011$). On the other hand, an analysis of mean PSI-SF scores based on maternal education level showed statistically significant differences, particularly in the Total Stress (TS) ($p = 0.043$) and Parent-Child Difficult Interaction (P-CDI) ($p = 0.018$). Post-hoc analysis showed that secondary licensed mothers scored significantly higher than high school licensed mothers on the Parent-Child Difficult Interaction (P-CDI) scale ($p = 0.043$). Mothers with a middle school diploma score significantly higher than mothers with college education on the Difficult Parent-Child Interaction (P-CDI) ($p = 0.005$) and Total Stress (TS) ($p = 0.007$) scales.

DISCUSSION

In agreement with previous studies, the results of this survey provided further evidence that the confinement measures and changes in daily routine imposed by quarantine negatively affected the behavioral and emotional dimensions of both children and parents (Saurabh & Ranjan, 2020), causing a significant increase in parental stress (Cusinato et al., 2020; Marchetti et al., 2020), mainly linked to feelings of inadequacy with respect to one's role in such a delicate situation and concern for the future (Coyne et al., 2020). Consistent with previous literature, the results of the present study also suggest that children and adolescents with neuropsychiatric disorders have a greater difficulty in adapting to large changes occurring in the lockdown period than typically developing peers, and this further stress parenting (Cusinato et al., 2020; Colizzi et al., 2020).

The suspension of teaching activities in the presence and the interruption of rehabilitation programs weighed on the parents, who lost the support of school services, therapists and ASL, with significant consequences, especially for the families of patients with Autism Spectrum Disorder (Drogomyretska et al., 2020; Colizzi et al., 2020). Many rehabilitation centers have had great difficulty in adopting the telerehabilitation services recommended by the Istituto Superiore di Sanità (ISS, n.d.) and the Italian Society of Child and Adolescent Neuropsychiatry (SINPIA). In fact, out of a sample of 271 children, only 28 continued the rehabilitation therapy in telematic mode. Furthermore, the limitation of medical visits to emergency cases weighed on parents' concerns about not receiving adequate assistance in case of need.

In this work it was possible to compare the data collected through the PSI (Parenting Stress Index) -Short Form to measure the level of parental stress with the results of the same questionnaire administered in the pre-Covid phase, verifying if there were variations in the level of stress that is often present in the parents of children with disabilities regardless of emergencies such as lockdowns.

This comparison revealed an increase in scores in all scales of the questionnaire, with statistical significance in the Total Stress (TS) and in the Parental Distress (PD) subscales. These results suggest that the greater level of parental stress is mainly related to the parent's difficulty in perceiving their own adequacy to the parental role and only marginally to the characteristics of the child or the parent-child interaction.

Furthermore, the parents indicated as critical factors the concern for the situation (high in 53.35% of cases and moderate in 33.95%) and the greater difficulties in managing the child (high in 26.94% of cases and moderate in 25.09%).

In 47.49%, worsening of clinical symptoms was reported, which in 12.18% was severe (of these, 31.04% were parents of children with autism, 46.2% with other neurodevelopmental disorders and 22.7% with epilepsy). The 47.61% of parents observed an increase in externalizing problems in their children, such as aggression and irritability: 19.19% in the mild form, 16.6% in the moderate form and 11.81% in the severe form. The increase in internalization problems in children, linked to the lock-down situation, was noted by 20.3% of parents; in 8.12% of cases the increase was mild, in 8.49% it was moderate and in 3.69% it was severe.

Among the socio-demographic variables considered, both the age of the parents for the parental anxiety subscale (PD) — as the age of the parents increases, the perception of parental stress increases — and that of the child in the Parent–Child Interaction Difficult subscale (P-CDI) — higher ages correspond to higher dysfunctional interaction scores — seem to influence the questionnaire results.

The paternal level of education did not give rise to statistically significant variations in the different PSI-SF subscales, unlike the mother's educational qual-

ification: a lower level of education (middle school) was linked to significantly higher levels of stress compared to a higher education level; in particular in the Total Stress (TS) and Disfunctional Parent–Child Interaction (P-CDI) scales.

CONCLUSION

Finally, the study shows that only 1.11% of the respondents declared that they had used, or that of another family member, the online psychological support services available to the population.

The results of this study provide evidence that changes during lockdown adversely affected the behavioral and emotional aspects of children with neuropsychiatric disorders, and parents with significantly increased parental stress. It could be useful to encourage, through adequate information and an awareness campaign, the use of online psychological services to support the psychological well-being of parents in this difficult period.

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DELAYED CUTANEOUS MANIFESTATIONS IN PATIENTS WITH COVID-19 CAUSED BY SARS-COV-2. CLINICAL OBSERVATIONS IN THE RUSSIAN FAR EAST

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ABSTRACT — Chung M. K., Zidar D. A., Bristow M. R., et al. (2021) warn that a historic pandemic due to the coronavirus disease, COVID-19, could have negative potential consequences for the cardiovascular system for millions of survivors worldwide [1]. At the present stage, mechanisms of the long-term consequences of COVID-19 disease caused by SARS-COV-2 are not studied well; any assumptions regarding to the consequences of SARS-Cov-2 are hypothetical. Therefore, long-term effects require in-depth research in the dynamics of recovery process after severe infections and rehabilitation of survivors. This will facilitate and increase the effectiveness of the treatment protocol for this disease and its complications [2]. The duration of the incubation period of the disease, the presence of sometimes not expressed or absent symptoms makes identification of the disease caused by SARS-CoV-2, COVID-19 infection, in some cases, too complicated [3]. The duration of the rehabilitation time, the complexity of pathogenetically based treatment do not yet have comprehensive information, therefore, it is difficult to resolve the issue of the timing of vaccination of patients who have recovered from COVID-19 infection. All the data obtained for each case of the disease, depending on age, the presence of concomitant diseases and individual terms of health recovery with the characteristics of complications in the late periods after recovery, has high relevance and great importance [4]. According to Wu Y., Guo C., Tang L., (2020), the excretions of recovered patients remains dangerous for others around 5 weeks after clinical recovery [5]. At the moment, there is practically no exact information about the duration and intensity of immunity in an infection caused by SARS-CoV-2 [6]. To develop protocols of COVID-19 treatment, principles and paradigms for the development of alternative effective vaccines for the prevention COVID-19 affects the health of consumers of the vaccine, which requires long-term monitoring in the post-recovery.

KEYWORDS — COVID-19, SARS-COV-2, SARS-COV-2 complications; skin rashes, endothelial dysfunction, thrombosis, fibrosis of the lung tissue, cognitive abilities, asthenia, "Covid nails".

INTRODUCTION

The analysis of the long-term consequences of COVID-19 infection in patients of the Primorsky Territory (Russian Far East) with a severe form caused by SARS-COV-2 has been performed. The late and long-term consequences of the disease in recovered patients with COVID-19 were evaluated. It was found that some patients has got complications in the function of the organ of vision, increased blood clotting. Vesicular skin rashes are observed that developed after 6 months and persist for a year. On the skin of the extremities, small blood vessels are visible, as a manifestation of dermatological disorders, which are characterized as a looped vasculature due to the capillary damage. There are complaints of problems with the cardiovascular system in the form of a rapid pulse, tachycardia. Most patients with COVID-19 have had minor or mild neurological symptoms. Some patients complained of headaches, memory impairment, increased weakness and rapid fatigue. MRI studies have shown that not all patients got recovery of lung tissue, some of them shown persistent fibrosis. In the presence of a clinical picture of lesions of the skin vessels, a clinical blood test is required to prevent thrombosis and ischemia. Obtained data expand the understanding of the pathogenesis and skin complications of COVID-19, which is necessary to improve the skills of young dermatologists and infectious disease doctors. The data can serve as a platform for the development of pathogenetically based prevention of complications of COVID-19 infection caused by SARS-COV-2.

Aim of research

The purpose of our investigation was to study and analyze COVID-19 infection complications on the

skin caused by SARS-COV-2 in a long-term period after the illness.

METHODS

The study was carried out in the dynamics of patient observation (10 men and 9 women) of older age groups receiving treatment at infectious diseases hospitals in Vladivostok, Primorsky Territory, in strict accordance with the provisions of the Helsinki Declaration (2000–2013) based on informed consent of patients. The study includes analysis of the dynamics of distant clinical manifestations development in the skin, obtained after discharge from the hospital and in the process of rehabilitation measures. The patients were on outpatient observation with monitoring of clinical complaints for 1 year after the disease. We focused on skin clinical symptoms during the development of the acute stage of infection and distant post-infectious manifestations using clinical and biochemical blood tests both in the disease dynamics and during the rehabilitation period. We further examined the data of female and male patients, according to the time of the onset of clinical symptoms after the disease, the severity of the infection. The exclusion criterion was cases of COVID-19 without complications in the form of skin symptoms, both during the acute period of infection and in the long-term period after the illness. The exclusion group included patients who had pathology of the skin and its appendages before infection with SARS-COV-2. The distribution of patients by age group is shown in Table 1.

Table 1.

Age groups (years)	Number of patients examined (absolute number)	
	Women	Men
20–39	3	3
40–59	3	3
60 and more	3	4
Total	9	10
Total number of examined patients	19	

RESULTS

As a result of patients observation admitted to a hospital in a serious condition with diagnosed pneumonia and confirmation of SARS-COV-2 etiology, complications in the form of developed local skin lesions of the upper and lower extremities were established. The ratio of the number of hemoglobin molecules to the total number of molecules in conjunction with oxygen — saturation in the acute period dropped to 80%, which corresponded to respiratory failure of the 2nd degree. In addition to complex anti-inflammatory treatment, non-invasive mechanical ventilation, oxygen therapy, and anticoagulant therapy were used.

We found that in the acute period of the disease and the long-term period after the infection, patients of older age groups with a severe level of illness (with the use of artificial lung ventilation (ALV), there were complications in the form of skin rashes. Rashes resembling herpes, on the inner surface of the palms, were observed both during the disease and in 1 year after discharge from the hospital, (Fig. 1).

In addition, on the lower extremities, local manifestations of complications were characterized by small vessels with signs of circulatory disorders. The legs usually had blue, purple (more commonly) or red linear and arborizing telangiectasias (Fig. 2).

Subcutaneous thin telangiectasias were located both protruding above the skin surface and in depth, while they were expanded by more than 0.1 and 0.2–0.3 mm. The sizes correspond to the present disruptions in all structures of the microvasculature, both in capillaries, in arterioles and venules. We also observed polymorphism of telangiectasias: arachnids - with a clearly defined center and vascular rays radiating from it (common arterial); dendritic — with a branching, tree-like structure and linear, parallel-directed vessels.

Usually there are manifestations of reticular varicose veins, but they may also indicate about changes of the hormonal (mainly estrogenic) background. The same stars and nets can be on the hands, but much less often. With such clinical manifestations, it should be borne in mind that any systemic disease can disrupt the blood circulation in the microvasculature of the skin, which can subsequently manifest itself in the form of transient anomalies in their structure and appearance.

In the group of patients during observation, there were no signs of severe lesions of the skin and its appendages, such as *cob-like nails*. Rashes similar to skin diseases of unclear etiology were not detected.

DISCUSSION

Some of the pathological manifestations could be considered the side effects of exposure to ultraviolet radiation after treatment with glucocorticoids. Perhaps, the appearance of spotted or stellate telangiectasias is a consequence of ultraviolet intolerance (photodermatitis), after the use of dexamethasone.

In contrast to the symptoms of skin pathology upon admission to the hospital COVID-19, which may result from the use of drugs for the treatment of

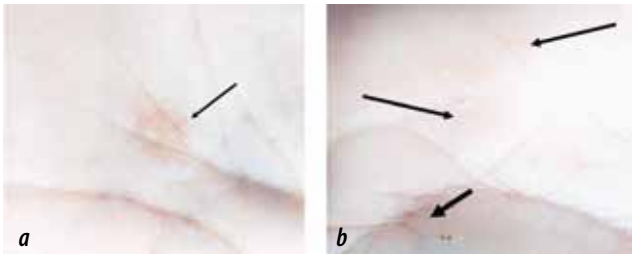

Fig. 1. 65-year-old female patient after complications of COVID-19 infection caused by SARS-COV-2 12 months after the illness.
a), b) vesicular rashes on the skin of the palms

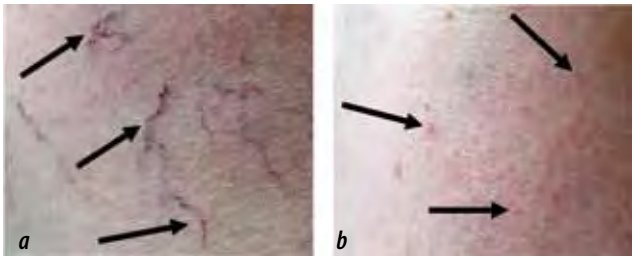

Fig. 2. 65-year-old female patient with complications of COVID-19 infection caused by SARS-COV-2 12 months after the disease.
a) capillaries of the skin of the outer surface of the thigh, telangiectasia in the form of branch-like reticules; b) the skin of the anterior surface of the leg, telangiectasia in the form of stellate mesh.

infection caused by SARS-COV-2, clinical signs of skin abnormalities in the patients appear after a few months or a year. This reflects the true state of the immune system and blood vessels of the skin, displayed without the use of medications.

Canedo-Marroquín G., Saavedra F., Andrade C. A., et al. (2020) argue that clinical symptoms may result from the migration of immune cells to the affected organs with an increased release of pro-inflammatory mediators that contribute to the development of the disease and make the immune response a major player during the development of pathological manifestations in the skin with COVID-19 [7].

Bernard I., Limonta D., Mahal L.K., Hobman T.C. (2020) note that despite the fact that COVID-19 is primarily a respiratory disease, extrapulmonary manifestations of COVID-19 include gastrointestinal, cardiovascular, renal and neurological pathology [8]. Recent studies have shown that endothelial dysfunction in COVID-19 can aggravate these deleterious phenomena, provoking inflammatory and microvascular thrombotic processes in the skin.

There is evidence that SARS-CoV-2 can infect endothelial cells by binding to the cell receptor for angiotensin converting enzyme 2 (ACE2) using the viral spike protein [9]. Accordingly, it is necessary to investigate the parameters that not only lead to severe illness in patients with COVID-19, but also to analyze how direct infection of endothelial cells with SARS-CoV-2 can further contribute to the process of circulatory disorders in various organs and skin [10, 11].

CONCLUSION

Due to the ongoing pandemic, new symptoms of multiple organ damage, including skin lesions, are emerging in the clinical symptoms of COVID-19. Monitoring studies and analysis of complications in recovered patients after infection with SARS-CoV-2 are needed, aimed at a better understanding of the epidemiology, the mechanisms of pathogenesis of COVID-19 infection caused by SARS-CoV-2. These screening studies will provide an understanding of the key issues of the pandemic impact, aimed at containing the outbreak of the disease and developing a timetable for effective preventive vaccination in recovered patients.

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THE CONSEQUENCES OF COVID-19 PANDEMIC ON DIET AND PHYSICAL ACTIVITY

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ABSTRACT — Aim: The COVID-19 pandemic has had major consequences on our habits and style of living. For this study, an online questionnaire was conducted online, with a total of 103 participants. This survey aimed to determine the pandemic's implications on nutrition and behavior patterns.
Results: During quarantine, the feelings of hunger and satiety did suffer changes: 17% of the cases reported a decrease, compared to the vast majority (55%), who experienced high levels of hunger. Three-quarters of participants reported either an enhanced or at least a similar intake of main meals and snacks between meals. Boredom and stress proved to be the central factors determining these results. When the participants were asked about the level of physical activity, a small number answered that the frequency of training grew. Meanwhile, the subjects who moved occasionally had more time to do it at home. Most of the participants (75%) stated that the length of leisure time was "more than sufficient".
Conclusion: As the pandemic is still ongoing, more research should be made on a larger population, to obtain more accurate results.

KEYWORDS — psychiatry, pandemic, Covid-19, eating behaviour, eating disorders, physical activity.

INTRODUCTION

The outbreak of the COVID-19 pandemic was a catastrophic event. The public health measures implemented, especially the mandatory quarantine, has had a huge global impact (Grigoras & Ciubara, 2021). They have interfered with our daily lives, changed our habits and way of thinking, and had negative effects on our physical, psychological and mental health (Luca et al., 2020a; Luca et al., 2020b). Previous studies have revealed a wide range of psychosocial repercussions on the population during the outbreak of SARS-CoV-2 infection (Ammar et al., 2020; Di Renzo et al., 2020a; Di Renzo et al., 2020b). On a personal level, people experienced the fear of getting sick or dying, feelings of helplessness, and stigma. In particular, the fear of one's health or of their loved ones, social distancing, and the forced quarantine disturbed the emotional and mental well-being of each individual (Loue, 2020; Miu, 2019; Onea, 2019; Sandu, 2020). Multiple factors led to inappropriate food choices (e.g. home orders, easy access to the kitchen, lack of exercise, anxiety, stress). Although some good habits rose, such as eating home-cooked food, the amount of consumed food was the one being compromised. Adequate nutrition is important especially during this time, taking into consideration that obesity, cardiovascular diseases, and diabetes amplify the risk of complications from SARS-CoV-2 infection.

This study aimed to objectify the factors contributing to the changes in eating behaviour and to collect data on the pandemic's impact on diet, physical activity, and mental health.

METHODS

This study was based on a questionnaire conducted on an online platform (Google Forms), available to any device with internet access, distributed on various social networks. Adults were encouraged to participate without any exclusion criteria other than age (it was mandatory to be over 18 years old).

The survey included 20 questions, structured in three different sections: (1) personal and general data (age, sex, background, level of education, profession); (2) anthropometric information (weight and height); (3) lifestyle changes in eating habits and psycho-emotional aspects caused by social isolation during the pandemic (e.g. anxiety, depression, sleep disorders, emotional eating, perception of diet and appetite control, the level of grocery shopping frequency). Non-completed surveys were not included. No names were requested, the answers were anonymous and confidential according to Google's privacy policy.

RESULTS

After the validation of the data, 103 participants successfully completed the online survey and were included in the study. The results show that the median age of the population was 35.92 years, age ranging between 18 and 82 years (18–30 years — 50%, 31–40 years — 30%, 40–50 — years 10%, 50–60 — years 25%, more than 60 years — 5%).

The female participants (67 cases) represented the majority of the population (65%). Nearly 80% came from urban areas and a high percentage attained a graduate (40.77%) and postgraduate education (34.98%).

Concerning the weight of the subjects, 48 participants were overweight (46.60%), 30 had a normal weight (29.12%) and 25 were obese (24.27%). In addition, 53% were married (55 cases), 69 individuals lived in a nuclear family (66.99 %), 25 came from an extended family (24.27%) and the remaining 9 lived in joined families (8.73%). Based on income, 17 subjects belonged to the upper class (16.50%), 41 (40,4%) to the middle class, and 45 (43.69%) participants had low salaries. The average BMI of the participants was 25 kg/m^2. The majority of the participants (57) reported a stable weight (55.33%), while 41 (37.86%) gained weight and 5 (4.85%) lost weight during the pandemic.

Through quarantine, the feeling of hunger and satiety changed for more than half of the population: for 16.50% (17 cases) they decreased, in 28.15% (29 cases) they remained the same and for 55.33% (57 cases) they progressed. An important part of the cases stated that they raised the consumption of vegetables (70 cases — 67.96%) and fruits (58 cases — 56.31%). However, most of the participants reported unhealthy food choices, leading to higher intakes of sweets (80 – 77.67%), fried foods (57 cases — 55.34%), fast food (50 cases — 48.54%), and carbonated beverages (35 cases — 33.98%).

Regarding the psychological and emotional aspects, a high percentage of the respondents declared to have felt anxious (85.43%) or depressed (51.45%) during the lockdown.

Overall, participants decreased their level of physical activity (65.04%) during the confinement. On the other hand, a part of the people who were practicing some kind of sport before the pandemic (14.56%) increased their training and fitness level. Furthermore, a few cases, who were exercising only occasionally, declared they spent more time doing physical exercises (10.67%). The most common forms of activity were jogging, aerobic exercise, and doing household chores. Additionally, 77 individuals reported they had more time to relax at home (74.75%).

DISCUSSION

The objective of the present research was to determine changes in eating behavior, physical activity, and psycho-emotional states, before and during the lockdown of the COVID-19 pandemic in an adult Romanian population. Our findings illustrate that even if healthier lifestyle practices, like eating more fruits and vegetables, were adopted, the frequency and the quantity of consumption were higher.

From a psychological and emotional point of view, data from this study support the conclusions of other studies conducted around the world during the lockdown (Baroiu et al., 2021; Di Renzo et al., 2020a; Isaacs et al., 2021; Konttinen et al., 2010; Malta et al., 2020; Moynihan et al., 2015; Stanton et al., 2020). These studies proved that the COVID-19 lockdown led to emotional imbalances and psychological disorders, affecting the population's overall wellness. The measures of staying indoors and working from home, the social isolation, and the incapability of close contact with other people caused elevated levels of stress, depression, and anxiety throughout the studied population (Di Renzo et al., 2020a; Konttinen et al., 2010; Stanton et al., 2020).

In particular, this study showed that the global intake of fast food, sweets, fried food, soft drinks sweetened with sugar significantly increased. Similar results were found in other countries (Di Renzo et al., 2020a; Di Renzo et al., 2020b; Malta et al., 2020; Moynihan et al., 2015).

Participants who were not used to doing sports before the lockdown did not consider this event as an opportunity to start. However, a small part of the cases, who already did some kind of physical activity, reported an improvement in the frequency and duration of training. Notably, the involvement in aerobic exercise and household chores was important.

The questionnaire used in this study had many strengths. It was easy, short, and did not require more than 10 min to be completed and submitted. Also, it provided critical insights on mental and emotional health and on lifestyle-related habits.

This study had some limitations like the small number of participants, the fact that the majority came from urban areas, the level of income, and the impossibility of long-term follow-up.

The coronavirus pandemic has caused many significant changes: improper diets, lack of exercise, and negative emotional background. Although some good habits have increased, such as eating home-cooked food or doing exercises in the house, the unfavorable aspects (stress, anxiety, depression, food addiction) surpassed the positive ones.

In conclusion, the online questionnaire had good reliability, making it a suitable tool in determining the changes that affected the population's health during this period. These findings are extremely valuable in preventing later health disorders and in raising the effectiveness of future therapeutic interventions.

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THE PSYCHO-EMOTIONAL IMPACT OF COVID-19 INFECTION ON MEDICAL STAFF IN THE FIRST-LINE MEDICAL INSTITUTION IN MOLDOVA

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ABSTRACT — The outbreak of the COVID-19 pandemic has substantially affected the lives of people around the world, especially after the World Health Organization declared a global pandemic in the second week of March 2020. The research and the clinical observations in this field show that, during the pandemic, many people display stress, anxiety or depression, which are fuelled by fear and the pathological worry of infection, of coming into contact with possibly contaminated objects or surfaces. It exacerbates the fear of strangers who might have an infection, the fear of socio-economic consequences and the symptoms of the traumatic stress associated to the pandemic. During the COVID-19 pandemic, the medical population proved to be susceptible to burnout syndrome - physical and emotional exhaustion, poor performance, indecision, fatigue, insomnia, anxiety, depression, all due to the increased work requirements, prolonged work, frustration in case of failure to save the patients' lives, pandemic-related uncertainty, fear of getting sick and infecting the family members. The frontline workers may face additional stressors during the COVID-19 outbreak: stigmatization of those working with COVID-19 patients; strict bio-security measures; physical pressure by the protective equipment; physical isolation which makes it difficult to ensure the comfort of a sick or suffering person; constant awareness and vigilance; strict procedures to be followed in order to prevent spontaneity and autonomy.

KEYWORDS — psycho-emotional impact, burnout syndrome, anxiety, depression, first-line medical staff.

INTRODUCTION

The COVID-19 pandemic is still very strong worldwide. The people are tired and need more support to face this situation. A mental health crisis is as important as any other healthcare crisis. It might be difficult to predict exactly when a crisis will occur and it can occur without any warning (Luca et al., 2020a). The World Health Organization (WHO, 2020) declared mental health as the second public health priority during the COVID-19 pandemic. A crisis can occur even when a family has a crisis prevention plan and has applied the techniques taught by mental health professionals (Radulescu et al., 2020). We all do our best to cope with, using the information and the resources we have at the time of a crisis.

The work of medical services and of their staff is affected (Grigoras & Ciubara, 2021). Given the risk of the significant increase in the number of mental health conditions, it is essential to underline the need for urgent investments in mental health services. The COVID-19 pandemic has impacted or blocked the main mental health services in 93% of the world's countries, and the demand for mental health care is higher, according to a new WHO survey. The survey was conducted in 130 countries. It gives the first global data about the catastrophic impact of COVID-19 on the access to mental health services and points out the pressing need for enhanced funding in this area. The frontline healthcare workers are at a particular risk of COVID-related psychological distress, because of the heavy workload, life-or-death decisions to be taken and the risk of infection. Healthcare workers In China have reported high rates of depression (50%), anxiety (45%) and insomnia (34%) during the pandemic, and 47% of healthcare workers in Canada have reported the necessity for psychological support. Fear and worry about one's own health and the health of the loved ones, about the financial situation or job or about the loss of support services; changes in sleep or in eating patterns; difficulty in sleeping or concentrating; worsening of chronic health problems; worsening of mental health condition; increased use of tobacco and/or alcohol and of other substances are among the most frequent consequences of stress during an infectious disease outbreak (Luca et al., 2020b).

It is now crystal clear that mental health needs to be treated as a core element of our response to and recovery from the COVID-19 pandemic, Dr Tedros Adhanom Ghebreyesus stated. *This is a collective responsibility of governments and civil society, with the support of the whole United Nations system. A failure to take people's emotional well-being seriously will lead to long-term social and economic costs to the society* (Baroiu et al., 2021). *The scaling-up and reorganization of mental health services*

that are now needed on a global scale are an opportunity to build a mental health system that is fit for the future, said Dévora Kestel, Director of Mental Health and Substance Use Department, WHO. *This means developing and funding national plans that shift care away from institutions to community services, ensuring coverage for mental health conditions in health insurance packages and building the human resource capacity to deliver quality mental health and social care in the community*.

METHODS

On 1st of June 2020, we conducted an online meeting with the team of mental health supporters (to promote the algorithms for the provision of psycho-emotional support for the medical staff) and an online training programme for the medical staff working in national/municipal/district hospitals on managing the psycho-emotional stress and mental health maintenance in the context of the pandemic of the novel coronavirus infection (COVID-19) was provided.

The training promoted several tools for assessing the burnout, the anxiety and the depression, chosen among those available and internationally validated, which were stored on Google drive and all the participants were requested to fill them out:

— Personal Data;
— Depression Test;
— Anxiety Test – TAG 7.

After the webinar on burnout, an email was drafted and all the counsellors further sent it to the specified addresses, informing about the psycho-emotional support services, and requested to fill out the questionnaire. Weekly psycho-educational support webinars for the medical staff on possible mental health problems and how to overcome them were conducted throughout six weeks.

RESULTS

As a result of the application of the Vocational Burnout Questionnaire to first-line medical workers, the following results were obtained: 137 out of the total number of people were receptive and accepted to participate. The Vocational Burnout Questionnaire has a minimum score of 24 points and a maximum score of 120 points; 24 points means that the person does not go through a vocational burnout and 120 points — that the person has a very high level of vocational burnout.

Out of the 137 respondents: 16 people do not go through a vocational burnout; 67 people face a low level of professional burnout; 38 people face a medium level of vocational burnout; 17 people — a high level of professional burnout. Sample distribution by residence environment: 70,8% — urban environment; 29,2% — rural environment. Sample distribution by age: 20–29 years of age — 12,4%; 30–39 years of age — 27%; 40–49 years of age — 20,4%; 50-59 years of age — 32,1%; over 60 years of age – 8%. Sample distribution by professional activity: 32,8% — doctors; 56,2% — nurses; 10,9% — other (human resources, accounting, technical assistance, etc). Among the surveyed medical population, 89.1% presented different levels of exhaustion, requiring a psychological and/or pharmacological intervention. 119 out of the 137 respondents work at the National Centre for Prehospital Emergency Care (CNAMUP) and 11 — at the Emergency Medicine Institute (IMU). Or, these 2 institutions which are in the frontline of COVID-19 expressed openness to the vocational burnout questionnaire. The other institutions did not have any reaction. We are preparing the informative materials, including short videos, simple techniques for the institutions and people who have not reacted to the burnout test and will send them by e-mail.

Working with the 40 community mental health centres (CMHCs), an instrument was developed and promoted to evaluate the work of the centres with the population and the medical staff in terms of provision of psycho-emotional counselling, coping with anxiety and depression and all professionals providing the above services were requested to fill out a questionnaire. 195 service provision questionnaires were filled out. Here are the reasons for service request: 62% (121 p.) — anxiety; 59,5% (116 p.) — signs of depression; 52,3% (102 p.) — asthenia, fatigue; 44,6% (87 p.) — occupational exhaustion; 40% (78 p.) — sleep disorders, etc. Only 10% of services were provided directly, the rest of them - through other indirect methods. 68,7% of interventions (134 p.) consisted in psycho-education, 36,4% (71 p.) — in motivational counselling, 29% (57 p.) — in prescribing medication and 13% (26 p.) — in psycho-therapy techniques. 79% of service requests were made by women and 21% — by men.

A training on starting the provision of psycho-emotional support for managers and medical staff working in hospitals all over the country in the national context of the COVID-19 epidemic was conducted for hospital managers countrywide. This training was attended by managers and persons in charge (key persons) of providing psycho-emotional support from 14 municipal institutions. Measures and actions needed to provide psychological support for infected people from the general population during the COVID-19 epidemic were developed. In order to improve the quality of recovery care provided to patients with COVID-l9 infection at all stages of healthcare, an

online training seminar on the implementation of the national guidelines was conducted: Medical Recovery of Patients with COVID-l9 Infection. After the launch of the WHO patient's guide — Recommendations for the development of self-care skills in the rehabilitation period after COVID-19 — this concept was used in the national guidelines on patient rehabilitation approved by an order of MHLSP. We subsequently drafted a letter to mental health services countrywide to launch this activity. An information and support campaign for people in the recovery period started in December 2020: "Support for the physical and psychological rehabilitation after COVID-19".

DISCUSSION

The coronavirus disease 2019 (COVID-19) pandemic may cause stress among people. Its repercussion, such as fear and anxiety about this new disease, can be overwhelming and give rise to powerful emotions in adults and children. Coping with stress in a healthy manner makes people and carers, as well as the community stronger. The obsessive-compulsive disorder seems to be one of the most serious long-lasting mental health issues related to the Covid-19 pandemic, according to the psychologists. They point out that, because of its unprecedented nature and scale, the coronavirus crisis brings another layer of uncertainty, if compared to previous financial crises. As a result, people who "are not good at dealing with uncertainty" or those who struggle to handle the situations beyond their control, can face an especially challenging situation.

Moldova and its population are quite special, as people in our country consider that suffering is part of us and that asking for help is somehow shameful, but the medical staffs needs to be supported in spite of this fact. Thus, we opted for different intervention methods: submission of the questionnaires with positive signs (+) of burnout to counsellors; sending to each person emails with the results and providing solutions, sending emails with a series of personalized support messages and links for the screening of depression and anxiety and recommendations, communicating with each person by email and receiving their possible requests.

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THE PRACTICES OF FAMILY-CENTERED CARE FOR HOSPITALIZED CHILDREN: COMPARISON OF IRANIAN MOTHERS AND NURSES' PERCEPTIONS

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ABSTRACT — Background: Family-centred care (FCC) practices are challenging from the perspectives of both parents and health professionals.
Purpose: This study aimed to compare experiences with FCC practices between Iranian mothers and nurses.
Design and Methods: This comparative cross-sectional study was conducted in 2019 on 233 mothers with hospitalized infants or children and 233 nurses working in neonatal intensive care units or pediatric wards selected using convenience sampling at five hospitals in Iran. Data regarding experiences about FCC practices among mothers and nurses was collected through the Perceptions of Family-Centered Care-Parent (PFCC-P) and the Perceptions of Family-Centered Care-Staff (PFCC-S) questionnaires containing the three subscales of Respect, Collaboration, and Support and 21 similar items. Descriptive and inferential statistics were utilized for data analysis using the IBM SPSS Statistics software. The significance level was set as $p<0.05$.
Results: The mean and standard deviation of mothers' and nurses' experiences of FCC were 2.68 ± 0.53 and 3.05 ± 0.39; respectively (range, 1–4). The lowest score belonged to the subscale "respect." There was a statistically significant difference between mothers and nurses in their experiences with FCC practices in all three subscales. The nurses reported more positive experiences with FCC practices than the mothers did ($p<0.001$).
Conclusion: The mothers and nurses exercised FCC differently and nurses' experiences were more positive. Reasons behind the difference in mothers' and nurses' experiences with FCC practices should be investigated. Interventions are required to remove barriers associated with FCC practices in accordance with healthcare in Iran.

KEYWORDS — family-centered care (FCC), child, hospital, mother, nurse.

INTRODUCTION

Family-centered care (FCC) is a key philosophy in pediatric nursing [1]. Accordingly, the patient and family are at the center of all healthcare decisions and their values, perspectives and choices of patients and their families should be respected [2]. FCC is considered a holistic care model, but its application in health care has been defined using various methods [3]. Nursing literature has confirmed the central role of FCC in the improvement of outcomes for both children and families compared to the traditional medical model. FCC may associate with improving health and well-being, greater efficiency, better communication, parents' empowerment in caring behaviors, parent satisfaction with services, better care experiences, greater perceived safety, reduced unmet healthcare needs, and reduced inpatient medical expenditures [4].

Parental understandings of healthcare service may also be associated with belief in the practice of FCC, cultural beliefs, provision of support for community members, support for family members, family roles, and complexity of the child's health conditions [5]. Family members may not feel ready to take full care of their child and refuse to participate in care interventions [6]. Another reason for diversities in the practices of FCC is that it is more associated with families and children outcomes rather than nurses' perspectives.

The mothers' and nurses' perceptions of the practices of FCC have been studied in various design and settings using different instruments. Smith and Kendal (2018) conducted a qualitative study on collaboration in the management of childhood long-term conditions from the perspective of parents and health professionals [7]. Both of them valued collaborative practices, but differed in their expectations of collaboration and adopted different mechanisms to foster it. In a study to explore how parents of preschoolers with cerebral palsy experienced the level of family-centered services, Myrhaug, Jahnsen, Østensjø (2014) used the Measure of Processes of Care (MPOC) within primary health care in Norway [8]. Some results showed that among the five scales of MPOC including enabling and partnership, respectful and supportive, coordinated and comprehensive care, providing specific information, and providing general information, the both scales of respectful and supportive care, and coordinated and comprehensive care received the highest ratings.

While FCC is a concept well accepted in westernized countries, it is less so in developing countries cultures play major roles in determining how care is given by health professionals and perceived by parents [9]. Further research on FCC within western and non-western pediatric care areas are needed to build knowledge and practice [10]. In addition, the difference between the perceptions of families and health professionals about FCC can hinder its proper practices [11]. Therefore, this study was conducted to compare the mothers' and nurses' perceptions of the practices of FCC in a country located in the Middle East region, Iran. The study's hypothesis was as follows:

There is a significant difference between the mean scores of mothers' and nurses' perceptions of the practices of FCC.

METHODS

Participants and samplings

This comparative cross-sectional study was conducted in 2019 on mothers with hospitalized infants and children and nurses working in NICU and pediatric wards of 5 hospitals in an urban area of Iran. Four hospitals included just one or both the NICU and pediatric ward. One hospital was a large referral children's specialized hospital with a wide range of neonatal and pediatric diseases services including NICU, PICU, medical and infectious, neurosurgery, oncology and nephrology. Sampling was performed using a convenience sampling method. Each mother and nurse who was eligible and tended to participate, was selected and enrolled in the study.

Given alpha 0.05, beta 0.05, and the effect size of 0.30, the sample size was 233 nurses and 233 mothers. Inclusion criteria for the mothers were: ability to read and write, hospitalization of the child for more than 2 days, and physical and mental health based on their own reports because of mothers' perceptions. Inclusion criteria for the nurses were: the education level of higher than bachelor's degree, having at least one year of work experience in pediatric wards, and physical and mental health based on their own reports because of nurses' perceptions. It should be noted that in Iranian hospitals, because of the implementation of the Islamic principles and consideration of privacy matters, only mothers are allowed to stay with their children and only female nurses are employed for pediatric care.

Instruments

Two questionnaires were used to collect data on the demographic characteristics of the mothers and nurses. The demographic questionnaire for the mothers included 10 questions about age, the education level, number of children, residence place, duration of travel from home to hospital, difficulty of mother's presence in the hospital, presence of someone who cares for the child at home, age of the child at admission. The nurses' demographic questionnaire included 10 questions about age, marital status, number of children, education level, employment status, job title, experience, work shift, and work place.

To measure the mothers' perceptions of the practices of FCC, the Perception of Family-Centered Care-Parent (PFCC-P) and to measure the nurses' perspectives of the practices of the FCC, the Perception of Family-Centered Care-Staff (PFCC-S) were used. The questionnaires were designed by Shield and Tunner (2004) to compare the perceptions of the mothers and healthcare workers regarding the practices of FCC with the same number of items (20 items) [12]. The translation, cross-cultural adaptation, and psychometric testing of the both questionnaires in Persian language had already been conducted and published in an article in 2018. One item was added to each questionnaire and the number of items increased to 21 items after the designer's permission [13]. Items 1–6 were for the domain of respect, items 7–16 were for the domain of collaboration, and items 17–21 were for the domain of support. The four-point Likert scale was used from never (score 1) to always (score 4) and the mean score varied from 1 to 4. Based on judgment, the mean score of 1 to 2 was considered as a poor level of practices of FCC, 2.1 to 3 as an adequate level, and 3.1 to 4 as a good level.

Two items for support domain in PFCC are: "When I come to the hospital I feel welcome" and "I am able to be with my child during procedures". The same items for this domain in SFCC are: "When parents come to the hospital they are made to feel welcome", and "Parents are able to be with their child during procedures".

Two items for collaboration domain in PFCC are: "When decisions are being made about my child's care the staff includes me", and "I understand the written material that has been given to me". The same items for this domain in SFCC are: "When decisions are being made about their child's care, parents are included", and "Parents can understand the written material that has been given to them".

For the third domain, support, two item examples in PFCC are: "The staff listens to my concerns" and "The staff understands what my family and I are going through". The same items in SFCC are as follows: "Staff listens to parents' concerns" and "Staff understands what the parent and their family are going through".

For test-retest reliability, the questionnaires were given to 20 mothers and 20 nurses who were not in-

cluded as the study participants, twice within 2 weeks. The intraclass correlation of the PFCC-S and PFCC-P questionnaires were 0.88 and 0.80, respectively. The Cronbach's alpha coefficient as an internal consistency for both questionnaires was obtained at 0.85.

Data collection and analysis

The author of this study as a woman allowed entry into the field and visited the pediatric wards. She referred to five hospitals affiliated to Shahid Beheshti University of Medical Sciences at different times and days of the week and asked the eligible participants to fill out the questionnaires. Since the main research site was the children's specialist hospital with different pediatric departments, about 60% of the participants of each group (mothers and nurses) recruited from that site.

Sampling was performed in a manner that the equal number of mothers and nurses entered the study through independent sampling means that the nurses were not necessarily working with the children of the mothers in the study. Data was analyzed using descriptive and inferential statistics via the SPSS software version 18. The normality of data was assessed using the Kolmogorov-Smirnov test. The significance level was set as $p< 0.05$.

Ethical considerations

This study was carried out after the approval by the Ethics Committee of Shahid Beheshti University of Medical Science. Permissions were obtained from the hospitals' authorities prior to the study. Before data collection, the purpose and methods for the study were explained. The informed consent form was signed by the participants. The questionnaires were completed anonymously.

RESULTS

The mean and standard deviation was 30 ± 2.8. Also, 69.1% of them had an education level of high school or university. They mostly (46.6%) had one child and 43.78% had two children. Also, 92.27% of the mothers lived in the urban area and 46.78% of the mothers experienced moderate to severe difficulty to attend the hospital.

The mean and standard deviation of the nurses' age was 32.5 ± 4.2. Most of nurses (91.42%) had a bachelor education degree in nursing and the rest had master degree. 81.54% had a work experience of less than 10 years.

The mean score of the mothers' and nurses' perceptions about the practices of FCC was 2.68 ± 0.53 and 3.05 ± 0.39 respectively. In all domains and the entire FCC, the nurses obtain higher scores.

The Kolmogorov-Smirnov test confirmed normality of the data. The mean and standard deviation of the mothers' and nurses' perceptions about the practices of FCC was shown. We found a significant difference between the mothers' and nurses' perceptions about the practices of FCC. There was a significant difference between the perceptions of the groups in the respect domain ($p< 0.001$). The mean score of the nurses' score for this domain was higher than mothers (2.64 ± 0.50 and 2.41 ± 0.49, respectively). A similar result was reported for the second domain as collaboration, and a significant difference was reported between the perceptions of the mothers and nurses ($p < 0.001$). The mean and standard deviation of the mothers' perceptions of collaboration was 2.83 ± 0.66, for the nurses it was 3.28 ± 0.46.

The independent t-test showed a statistically significant difference between the mothers' and nurses' perceptions about support ($p < 0.001$), indicating that the mean scores of nurses were more than the mean scores of mothers (3.10 ± 0.51and 2.52 ± 0.69, respectively). There was a statistically significant difference between the mean score of the mothers' and nurses' perceptions of the practices of FCC ($p < 0.001$) as the mean score of the nurses (3.05 ± 0.39) was higher than mothers (2.68 ± 0.53). Accordingly, the hypothesis of the study was confirmed as the mothers' and the nurses' perceptions of the practices of FCC in all domains had differences.

DISCUSSION

The aim of this study was to compare mothers' and nurses' perceptions of the practices of FCC in an urban area of Iran. Accordingly, the nurses' perceptions of the practices of FCC in the domains of respect, collaboration, and support were different from those of the mothers and the nurses reported higher scores. Contrary, Gill et al. (2014) stated that parents had a more positive perception of the practices of FCC compared to staff [14]. A qualitative study by Foster and Whitehead (2017) showed a difference in the perceptions of parents and health professionals regarding the practices of FCC [14]. In that study, the parents' perceptions were presented in terms of family, treatment, and communication and the health professionals' perceptions were family and treatment, indicating that parents emphasized more on communication. In a study on the implementation of FCC in care homes for children with neurodevelopmental disabilities, parents reported the weakness of FCC to respond to family needs, coordination, follow-up and support, and social resources [15]. Hill et al. (2018) found that parents identified unmet needs for the practices of the different aspects of FCC such as respect and dignity,

information exchange, and participation [16]. The findings of this study showed that the practices of FCC were not fully implemented from the perception of mothers and nurses. Similarly, in Saudi Arabia despite the confirmation of the importance of implementing FCC, nurses reported that this care model was not fully implemented in practice [17]. The health professionals in the Dall'Oglio et al. study (2018) reported that there was a significant gap between the perceptions of necessary and daily practices of FCC 9. Contrary, Gill et al. (2014) in a similar study in two large Australian hospitals reported that parents and staff reported satisfactory and good results of the practices of FCC. In another study in different European countries, all parents and nurses rated the practice of the FCC in the NICU high quality [14].

Reasons for not being complete practices of FCC could be a lack of education or awareness, fear of noncompliance with the family and its misconduct, lack of adequate knowledge of community resources for the fulfillment of family needs, beliefs and perceptions of employees of FCC and its potential benefits, lack of power of health professionals, low social support, lack of time, lack of resources, or insurance required for the implementation of FCC [18]. In addition to the above-mentioned barriers, communication issues, cultural barriers, and hospital policies could be other causes in the Iranian context. The mean scores of the mothers' and nurses' perceptions of the domain of respect were lower than the two other elements of collaboration and support. Conversely, Gill et al. (2014) reported a higher score for respect in parents and healthcare staff than other domains [14]. Respect means giving value to the cultural diversity of families and considering it a unique element. In the present study, the domain of respect consisted of parental greeting, the treatment of parents as parents and not typical visitors, respecting for the child and parent privacy, and the permission to stay with the child. Therefore, in comparison with other two domains, both mothers and nurses achieved a lower score on how the staff behaved with mothers and their children.

STRENGTHS OF THE STUDY

One of the strengths of this study was data collection using international questionnaires which had been already studied in term of psychometric properties in Persian language. The second strength point was to investigate the perceptions of mothers and nurses of the practices of FCC in a country in the Middle East which revealed the differences between the perceptions of the two groups about the practices of FCC.

LIMITATIONS OF THE STUDY

Given the fact that the study was carried out at a referral hospital in Iran, its results cannot be generalized to other pediatric wards in Iran. According to the authors, a limitation of this study was the effect of maternal fatigue and worries due to illness and child care and the nurses' fatigue due to workloads on the data collection. In addition, due to no presence of fathers in the pediatric wards, their perspectives were not assessed. The self-report identity of the data collection could affect the validity of the collected data. Therefore, observational studies on the practices of FCC are suggested.

Recommendations

Since the practices of FCC is not complete from the perspective of mothers and nurses, there is a need to improve the practices of FCC through modifying related policies in pediatric wards, provision of free visits for all family members, education and modification of nurses' beliefs about the importance of FCC and its dimensions, determining the role and extent of mothers' participation in FCC, education of nurses based on the continuous evaluation of family needs and aligning their understandings with those of mothers.

CONCLUSIONS

Considering FCC is a western healthcare model, it may not fit with all aspects of the health care culture in Asian and Middle Eastern countries. Therefore, more quantitative and qualitative studies on the implementation of this care model and its cultural adjustment are needed. Further studies on the factors associated with the low practices of FCC and differences in the perceptions of the mothers and nurses are required

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STORING MORAL IDENTITY AS A CORE OF THE PROFESSIONAL IDENTITY FOR FUTURE NURSES

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ABSTRACT — Evolving in time, the nursing profession construction started based on moral values, which were underlined by Florence Nightingale (1989) and it was associated with compassion and care, but with the technical development and increasing the complexity of care provided, nursing went from a job to a profession, which asks more and more technical competency and more and more pragmatism (Serra, 2008), losing in time the humanity part of the care, overwhelmed by the detachment discourse which became dominant in the times of an evidence based medicine (Charon, 2016; DasGupta, 2014). Going back to humanity became one of the main concerns of the researchers in the last decade and that put the focus on developing the interactional capacity of the students in nursing (Benner et al., 2009; Charon, 2016; Fagermoen; 1997; Gold, 2020; Sharpless et. al., 2015), one of the most important skill to develop being attentive listening (Charon, 2016) in the relation to the patient and the colleagues, attitudes promoted with the Narrative Medicine Program (NMP) conceived by Charon (2016), who is a doctor and professor at Medicine Faculty in Columbia University in United States.
Conclusion: The data resulted from this study suggests the need to direct the curriculum on the professional identity of the future nurses' development, which is in the same line with other studies, but also the need to help the students in the management of the experiences from their clinical practice considered significant to them, which could sustain the development of a professional identity guided by care and focused on the relationship with the patient.

KEYWORDS — professional identity, moral identity, narrative medicine, narrative moral identity, narrative nursing.

INTRODUCTION

This article is addressing the nurse identity formation from a social constructionist perspective. The context of the medicine development has an influence on what can be a good doctor or a good nurse. When promoting technical skills, a good nurse is a nurse who is very well technically trained and able to make pragmatic decisions and when promoting the care discourse a good nurse is a nurse able to apply medical procedures with empathy and being oriented to care for the patient. There are two ways when talking about care: to *care for* and to *care about* without confusing care with caregiving (Noddings, 2017). Looking from care discourse perspective a good nurse would be a nurse who *cares about* the one who *cares for*. Noddings (2017) tries to conciliate both discourses, considering there is a false problem, the nursing profession living somewhere in-between detachment and involvement and he suggests the virtue discourse which can be more appropriate for a medical profession which involves both care and knowledge.

Other studies also underly the need to develop both the technical and the human skills at their best in order to be a good nurse (Bliss et al., 2017; Popovici, 2013) only that sometimes these two discourses seem to confuse the students, especially when we are talking about the students in nursing in their first years of studies who does not have the ability to manage them both.

Using the stories that students in nursing are telling from their clinical practice the aim of the study is to identify what contributes to the development of the nurse professional identity and how the stories they tell can contribute to their sense of being nurses.

THEORETICAL FRAME

A social constructionist approach on nursing professional identity development

It is already known that the social constructionist view is about the way that people understand reality, which is seen as constructed through negotiation in daily life interactions of the people (Berger & Luckman, 1966; Sandu & Unguru, 2017). When it comes to identity, the social constructionist view is explaining it also as a construct of reality built in interaction with the others, a dynamic reality which is developing through social interaction (Gergen, 1994; Raskin, 2002) and which can determine social action. The medical context seems to have a lot of influence on people, because it touches always core values, being linked to high moral standards (Nightingale, 1989) since the beginning of the nursing profession what brings us to the discourse of care in medicine.

Rita Charon (2016), who is a doctor and a professor in medical school in Columbia University, empha-

sized the need to go back to humanity in medicine and to go beyond the detachment discourse which made the professions in medicine to lose contact with the patient. She started the NMP focused on developing attentive listening for the students in medicine and nursing in order to establish better relationships with the patients (Charon, 2016). The need for more humanity in medicine was noticed by many other researchers (Benner et al., 2008; Chen, 2015; Gold, 2020; Moscheta, 2020; Mueller et al., 2003) who considered that a nurse needs to have skills to manage the emotional aspects involved in practice to reach a high level of professionalism and competency in nursing. Between the two discourses met in medicine, to be involved or to be detached in practicing nursing, the moral dimension of the profession was always recognized, professional identity development being linked to the moral development as a condition for a nursing practiced with care (Chen, 2015; Ranjibar et al., 2016).

How stories get meaning

People are storytellers because everybody relates significant events from their lives to the others through stories (Rappaport, 1995; Robertson & Clegg, 2017). People are telling stories and they are storing themselves (Gergen, 2005), and students in nursing, while telling nursing stories to the others, are storing themselves like nurses and the way they are doing it can contribute to the development of their professional identity. The notion of story is linked to identity construction, identity being the result of stories and narrations, which allows people to understand and to know the reality (Somers, 1994). McAdams (2011) defines *narrative identity* as an *internalized and evolving story of the self that a person constructs to make meaning out of his or her life* (McAdams, 2011, p. 99). If we see the meaning like a construction of a *socially negotiated reality* (Sandu & Unguru, 2017) the story by which people gain meaning is a construct built in social interaction which will also influence peoples' communicative actions, including the stories they will construct in daily interactions. People are influenced by and are also influencing the communities' stories construction (Gergen, 2005) promoting a negotiated reality for community. Gergen (2005) considers that the discourses from a community underly a certain type of being which are promoted and sustained detrimental to others, which influence the self-definition of a person through narrative construction, which can be inter-relational validated if they are recognized as discursive descriptions socially available which can motivate the individual actions.

Churchill (2015) and McDonald (2009) attributes to Nightingale this quote: *Observation tells us the fact, reflection the meaning of the fact*. (McDonald, 2009, p. 723), so the facts that students are observing during their clinical practice can get meaning by reflecting on them. The meaning the students in nursing are getting from their stories may depend on the context they bring the facts to *reality*, if they talk to friends or to colleagues, for example. When telling stories, people will tend to describe them as good people, in our case, good nurses, rather than bad persons or bad nurses. Being good or bad depends on social discourses, including the professional discourses which involves moral aspects, also derived from the cultural background and the social context. Sandu et. al. (2020) define value like a *social construct resulting from the act of communication, as a negotiation of interpretations that individuals attach to elements of reality* (Sandu et al., 2020, p. 106) and that would give, to what it means a *good* nurse, flexibility sustained also by understanding "*caring, as described in care theory, pointing to the reciprocal quality of a relation,* (...) *not merely a set of prescribed acts*" (Noddings, 2017, p. 184).

Choosing a way of being is emphasized by Charmaz (2006), who investigated the identity development in chronically ill people. She describes four levels of identity, depending on the illness and disabilities, and people are challenged to choose either to diminish their identity goals or to make ambitious identity goals, moving from one level to another up or down (Charmaz, 2006). These identity goals are called *preferred identities*, because it helps the person to adjust her/his identity to her/his needs. These preferred identities are distributed in a hierarchy where the first one is "*the supernormal social identity*" (Charmaz, 2006, p. 124) associated with great accomplishments, "*the restored self*" (Charmaz, 2006, p. 124) connected to a self which existed before the illness, "*contingent personal identity*" (Charmaz, 2006, p. 124) a possible identity and "*the salvaged self*" (Charmaz, 2006, p. 124) which refers to the retention of a past identity linked to a valued activity or attribute, when the person becomes physically dependent.

METHODS

Students in nursing from a postsecondary school were invited to participate to a NMP held once a week during their clinical practice, where they were able to share with their colleagues the most significant stories. What was a significant story was defined by the storyteller. The participants were asked to give a written consent about the conditions of the meeting and their right to quite any time. The data were analyzed using Grounded Theory and there were thirteen interviews, lasting from 58 minutes to 120. There were 53 participants from 19 to 51 years, from the first till

the last year of studies and their participation was voluntary.

The interviews were based on a semi structured interview using outsider witness practice from the narrative approach.

RESULTS

As a secondary analyze of the data collected from stories from NMP, adjusted from the model provided by Charon (2016), the study underlined the dominant stories which are contributing to the start of the professional identity construction of the future nurses. Trying to reach the goal of being a good nurse for students in nursing can be challenging for their personal identity and thinking about this process like a movement inside identity levels (Charmaz, 2006) determined by facts observed or experienced in the medical field and in interactions with the patients and their colleagues, we can also understand the choices of the students for certain personal identities in nursing profession like intentional identities (*identity goals*, Charmaz, 2006) which can be valued or not, depending on the feedback from the patient. The identities which prove to be valued can become preferred identities and if not valued can come into focus of the student to be adjusted to the level the students feel comfortable. For example, the need for knowing more about the profession can be an intentional identity and experiencing what it means to know more about nursing in the relationship with the patient can make it a preferred identity and put the student to act in the sense of getting more knowledge. For example, for a student in nursing not knowing was seen as a moment of crisis:

That was the crisis. When you actually realize that you need to learn more, to get more information and to ask more questions in case that something is happening. (I048)

So, being in a moment of crisis can challenge the student identity and push him to find a preferred identity, which can be one of learner moving down in the identity hierarchy, but being a learner can be adaptative for the moment even if this can be seen like a step back from the professional identity of a nurse.

And also, if a student realizes that he/she can't manage some situations technically because of the lack of experience, he/she can go back to a lower level of nurse identity, like being only a student and that can make him/her feel better with the situation while trying to do as much as he/she can or to compensate on the moral actions where he/she feels confident and where can move up to his/her moral identity.

I mean, each one had an important moment, but I don't know, I guess lighting the candle meant...to be lightened and to...I don't know, (...) *To be honored...I don't know, at least a minimum of...aaa, the patient last wishes.* (...) *I don't know, it just came out* (*n.a. the idea of lighten the candle*)*...even this thing with the light it was just a thought...came out spontaneous just to lighten the candle, I didn't even need to think about what should have been done or.... I was the only one who...*(I030)

So, it would be hard to identify certain ways of being good nurses, considering the fact that this concept, seen like a value, is a *social construct resulting from the act of communication* (Sandu & Unguru, 2017), in relationship with the patient (and other colleagues) and even the dimension of care has its' own mobility being defined by the quality of the relationship (Noddings, 2017), underly the importance of developing good skills of communication and interaction so the nurse would be able to adjust on expressing being a *good nurse* in relation to the patient's needs (Fig. 1).

DISCUSSION

Charmaz's model of a hierarchy identity (2006) makes sense for the students in nursing identity either, their significant stories reported from the clinical practice suggesting the idea of building their identity moving from a level to another depending on the challenges they encounter for their sense of being as humans or as nurses, strengthening some preferred identities in some moments which proved their efficiency in the practical interactions with the patient and others in moments where other identities makes sense for the students actions and decisions.

The results of this study are in contradiction with other studies' results which sustain that in the first years of studies the students are more focused on the technical skills because they are oriented to learn more and after gaining the technical skills, they become more interested in the moral aspects of the profession (Benner, 2004). These differences can be explained by the fact that the participation of the future nurses to the NMP was voluntary so, they might have a strong motivation to becoming nurses and probably also high moral standards before joining the nursing studies, so personal values could have been a platform for their professional identity construction (Poorchangizi et al., 2017). Considering the moral development of the students during their formal education in nursing (Dukett et al. 1997; Riesch et al., 2000; Lin et al., 2010; Ranjbar et al., 2016) and the results of this study, we can recognize the important of the moral aspects involved by the nursing profession (Ranjbar et al., 2016). Using the model of identity hierarchy described by Charmaz (2006) the preferred identity coming from the students' stories is moral identity, which we can call narrative moral identity, being the result of the stories students brought from their clinical practice. The

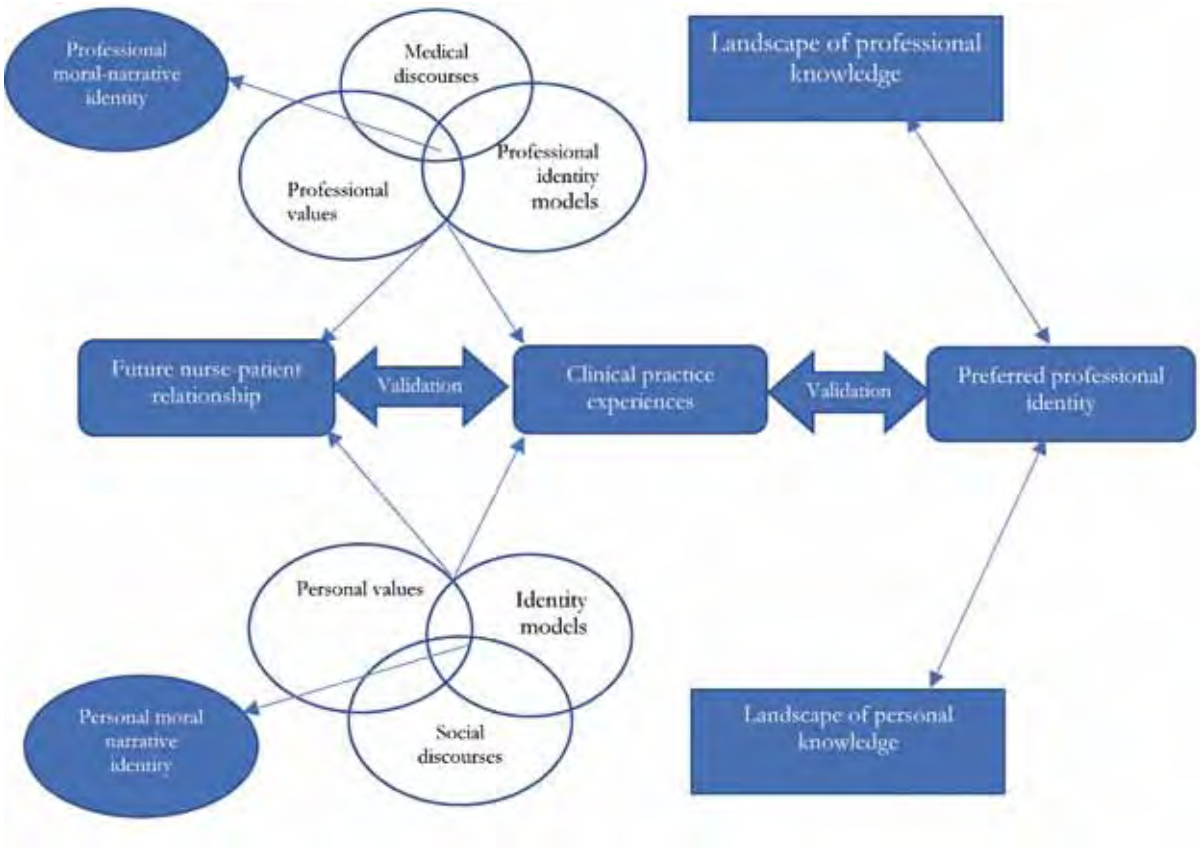

Fig. 1. *Nursing professional identity development model*

narrative moral identity seems to be the identity level preferred by the students participating to PMN, evolving on a continuum passing from the personal moral identity to the professional moral identity helping the students to get sense of their actions from a moral perspective and constructing goal identities for the technical aspects which seemed to put them one step back from the nurse identity. This process of developing the professional identity is influenced mainly by the patient–future nurse relationship which validates the future nurse identity as moral and skilled nurse, and making sense of the experiences from clinical practice which asks the student to move from different levels in the identity hierarchy depending on his needs for meaning, promoted by his clinical experiences. The educational context (which involves the personal and professional models of identity, the knowledge and the social and professional discourses) can offer the students a platform for nursing identity development where some preferred identities can be forged by growing clinical experience as a nurse and can, probably give more stability to the identity hierarchy, based on the moral identity, but these aspects can be explored in future studies. It is also to explore in future studies how people with different moral standards develop their professional identity and if the moral identity remains the core of their professional identity as nurses and how it changes along the studies and their professional experience.

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THE INFLUENCE OF SOCIAL DETERMINANTS ON HEALTHCARE CONSUMPTION IN WOMEN

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ABSTRACT — Noncommunicable diseases provide a significant impact on global health. Information about the factors influencing the noncommunicable morbidity and the need for medical care is important for forecasting need and distribution of healthcare resources. The article presents the results of a study dealing with the relationship between social determinants of health and acute conditions requiring medical care in women. Social determinants including the standard hierarchy of values and the level of education (high school) influence on individual self-assessment of health and are associated with lower need for medical care due to acute non-communicable diseases. The graph method was used for the study and can be proposed for further investigation of the influence of social determinants on the need for medical care.

KEYWORDS — social determinants of health, public health, self-assessment of health, life values.

BACKGROUND

The modern concept of prevention has been developed in relation to infectious pathology is not always adequate in relation to non-infectious pathology [1, 2]. The search for risk factors and determinants of health influencing the development of noncommunicable diseases is currently an urgent task of health and healthcare management [3, 4]. Social determinants of health, including life priorities (hierarchical structure of values) significantly influence on individual lifestyle and are of great importance in the healthcare management system. Social determinants of health influence on individual health indirectly mediating through the risk factors. The most important are the individual lifestyle and the features of lifestyle that are closely related to the hierarchy of life values and priorities. Despite the importance and significance of life priorities and their indirect influence on the health and morbidity as well as the need for medical care and women's self-assessment and of health has not been studied in detail.

Objective:
to analyze the influence of life values hierarchy on the self-assessment of health and need for medical care in women of reproductive age.

MATERIALS AND METHODS

An anonymous survey was completed by 566 women aged 18 to 49 years. In total, seven priority options (values) were proposed to arrange according to individual hierarchy: *Family*, *Work*, *Education*, *Career*, *Health*, *Material goods*, *Spiritual values*. The leading priority (*value*) was the number one, *significant priorities* (*values*) occupied the positions from 2 to 4 and the *non-significant vital value* — from 5 to 7.

The standard structure of life priorities was determined by comparing the structure characteristic of the group as a whole and the individual hierarchical structure for each woman. If the ranking places of at least three life priorities coincided, then such a structure was considered as standard or approaching standard (the first subgroup). Otherwise, if the number of matches was less than three, the structure was considered as non-standard for this group and the woman was included in the second subgroup.

For each subgroup, the indicators of self-assessment of health, the weighted average frequency of acute diseases, the level of chronic morbidity, the intensity of consumption of medical care and the level of medical activity were calculated. Self-assessment of health, as a fairly objective indicator of health is a standard method for population health studies [5, 6]. In this study, the assessment was carried out on a five-point scale, where 5 points were the highest level of health, while 1 point was the lowest indicator of health, according to the self-assessment data. Data on morbidity were obtained from medical records.

RESULTS AND DISCUSSION

The analysis of the influence of the structure of life priorities (values) on the frequency of acute diseases provided the weighted average frequency of acute diseases as 0.64 diseases cases per woman per year. In this subgroup of women (the standard structure of life

priorities), there were no frequently ill women (more than 3 acute diseases cases per year), and more than half of the surveyed women (53.1%) did not get sick at all during the year preceding the study. The study indicated also the influence of education (high school graduates or not) on the morbidity in the group of women with a standard structure of life priorities. The risk of acute conditions was lower in the subgroup with high school graduates (1.13 versus 1.62 acute diseases per year). This fact indicates more significant impact of the level of education on the acute morbidity comparing to the hierarchy of life priorities (values). In the subgroup of women with a non-standard structure of life priorities, the weighted average frequency of acute diseases was twice higher. 5% of woman in this subgroup wee ill more often than 3 times a year, and only 35.7% did not get sick at all during the year preceding the study.

There were no significant differences between the subgroups in terms of the incidence of chronic diseases. In both subgroups, the incidence rate was quite low at the level of 31–31.2 per 100 women.

The frequency of requests for outpatient medical care was higher in women with a non-standard structure of life priorities (2.04 vs. 1.55 requests per year). There were no differences in the need for inpatient and emergency medical care in women with a standard and non-standard structure of life priorities, as well as differences in the intensity of use of emergency medical care (1.4 vs. 1.42 calls per year).

Women with a standard structure of life priorities indicated more responsibility and activity regarding preventive examinations procedures (28.1 vs. 9.5 per 100 respondents in each subgroup, respectively). Women with a non-standard structure of life priorities indicated less responsibility in this area (6.25 vs. 26.2 per 100 respondents in each subgroup, respectively). There facts demonstrate that individual prophylactics activities did not depend on the structure of life priorities. Approximately two-thirds of women from both subgroups indicated an early request for medical help when pathological symptoms occurred.

The influence of the structure of life priorities on the indicators of self-assessment of health was quite significant. The level of self-assessment of health was higher among women with a standard structure of life priorities, but the differences between these two groups were not significant ($p>0.05$). At the same time the level of education provided a significant impact on self-assessment indicators. In the subgroup without higher education, higher self-esteem indicators were found in women whose structure of life priorities was standard (3.54±0.13 points versus 3.29±0.09 points, the differences are significant, $p<0.05$). In the group of women with a high level of education, there were no differences in self-esteem indicators depending on the standard structure of life priorities (3.56±0.09 vs. 3.54±0.09 points, $p>0.05$).

CONCLUSION

The data obtained indicate that life priorities (values) and their hierarchical structure should be considered as a significant social determinant of the health of women of reproductive age due to relationship with acute morbidity, the need for healthcare services and self-assessment of health. There are no differences in the prevalence of chronic diseases in women, depending on the structure of life priorities. However, it is not advisable to consider life priorities and their structure as an isolated and independent determinant of health, which does not depend on other determinants. The structure of life priorities determines, along with other social determinants, a person's behavioral reactions, which, in turn, determine the way of life. According to the data obtained, the dependence of the structure of life priorities and the level of education is obvious, which gives grounds to consider not each determinant separately, but the existing complex of determinants, the severity of which is different in each age and sex group. In this case, an approach based on weighted oriented graphs can be used as a model of the influence of a group of determinants on public health indicators and the intensity of consumption of health services, and the use of scenario analysis methods is also relevant.

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QUALITY OF LIFE IN WOMEN OF REPRODUCTIVE AGE WITH RECURRENT GENITAL HERPES

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ABSTRACT — Background: Current statistics show that the highest incidence of genital herpes is observed among women of reproductive age. Since these patients have an active lifestyle, frequent outbreaks of genital herpes can cause physical and psychological discomfort.
Aim. To assess the quality of life of women of reproductive age with recurrent genital herpes.
Methods: The quality of life in women suffering from recurrent genital herpes for more than 5 years (n=86) was evaluated. The control group included healthy women (n=91) according to the data of external genitalia examination and PCR diagnostics. The Health Status Survey (SF-36) in points was employed to measure the health status of women in both groups.
Results: We established a significant difference between two groups of patients in terms of the influence of the following indicators on functional limitations: general health condition — 12.1±0.8 points; pain — 8.3±0.4 points; physical problems — 18,3±3,7; physical activity — 8.2±0.6 points ($p<0.01$).
Conclusion: Symptoms of herpetic infection and long-term persistence have an impact on the performance of daily tasks and the quality of life of the patient.

KEYWORDS — Genital Herpes, quality of life, pain, physical activity.

INTRODUCTION

Today, herpes simplex virus is one of the most common human viral infections [1]. According to blood serum studies, in 80% of the adult population, antibodies to the herpes simplex virus are detected [2], and in 6-10% of patients, herpetic infection is accompanied by clinical manifestations.

Over the past 10–15 years, the number of registered patients with genital herpes in Russia has increased 2–2.5 times, with the highest incidence observed in the age group under 30 years [3]. Often recurrent genital herpes presents a serious medical and social problem in women of reproductive age, as it poses a powerful psychotrauma that limits their social activities, worsens the quality of life, and affects a reproductive health.

Recently, the question of the quality of life in patients with recurrent genital herpes has become increasingly important [4]. It is known that this category of patients is concerned not only with the physical suffering from a relapse of the disease. The psychological, social and psychosocial impact of herpes on their lives is much more painful for these patients. Low self-esteem and self-respect, depression, anger, guilt, and sexual problems create a vicious circle for patients with genital herpes and significantly reduce the quality of life.

Aim: to assess the quality of life in women of reproductive age with recurrent genital herpes.

METHODS

We evaluated the quality of life in women suffering from recurrent genital herpes for more than 5 years (n=86), who made up the first group of the study. The second (control) group included healthy women according to the examination of the external genitalia and PCR diagnostics (n=91). We evaluated the quality of life of women in both groups using the Health Status Survey (SF-36) in points. Using the SF-36 questionnaire, we took into account the following indicators: physical and social activity; general and mental health; vitality; the role of pain, physical and emotional problems in life restriction (LR). Women who had genital herpes disease less than 5 years were excluded from the study, as well as those with a detected papillomavirus infection and other sexually transmitted diseases.

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

Women of both groups were comparable in age:

26.8+6.2 years in the first group, 24.6±5.8 years in the second group (p>0.05). The majority of women in the first group complained of burning sensation (90.6%), itching (95.3%), rash in the form of small bubbles (83.7%), dyspareunia (38.3%). The duration of chronic genital herpes in women of the first group was 9.2±3.1 years. A comparison of the quality of life in women of both groups according to the SF-36 questionnaire is shown in Fig. 1.

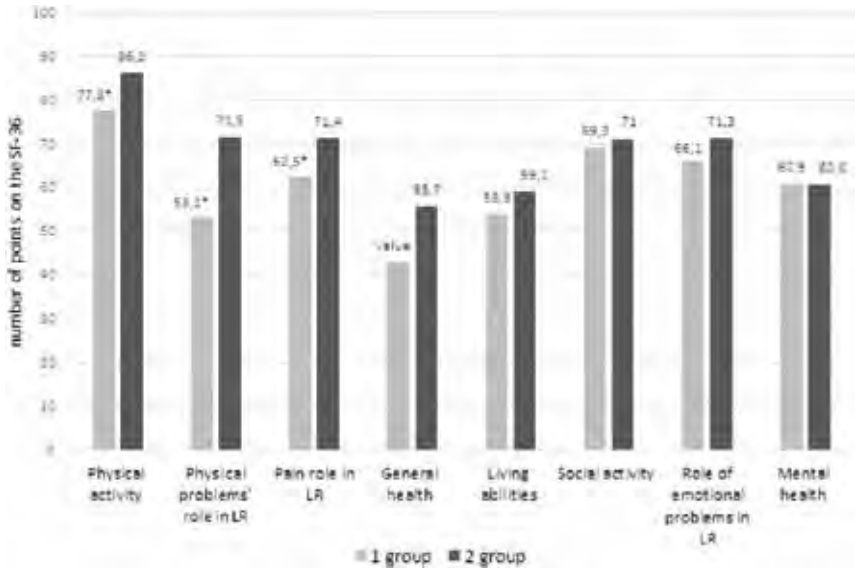

Fig. 1. *Comparison of the quality of life in women of both groups according to the SF-36 questionnaire*

** p <0.01 indicators of the first group in relation to the second.*

In patients who have recurrent genital herpes for more than 5 years, the greatest difference with the control group was in the parameter *the influence of physical problems on functional limitations*, which was 18.3±3.7 points (p <0.01). That is, there is a clear relationship between disease duration and quality of life (satisfaction with their physical abilities).

We also obtained a significant difference in the indicators of general health, the influence of physical activity and painful sensations on functional limitations by 12.1±0.8, 8.2±0.6, 8.3±0.4 accordingly. Consequently, the symptoms of a herpetic infection and the duration of the disease have an impact on the performance of everyday activities.

DISCUSSION

Recurrent genital herpes is one of the socially significant challenges in the practice of a gynecologist. Outbreaks of genital herpes can contribute to the development of chronic inflammatory urological diseases, and increase the risk of developing cervical cancer [3]. Moreover, the herpetic infection itself and persistent microflora contribute to the development of secondary symptoms: difficult and painful urination, the formation of chronic recurrent diseases of the lower urinary tract, up to the formation of paraurethral cysts and abscesses [2, 5, 6].

Also, many experts emphasise that in recent years, the percentage of people with depression and anxiety associated with chronic herpetic infection has increased. According to Royer H. R. et al., 95% of patients have depression, and 90% indicate concern about sexual disorders [7]. And, 15% of women believe that the infection with herpes may lead to death [7]. Some studies show that even primary serological imaging of the herpes virus may cause stress in patients [1]. Our observation demonstrates that the deterioration in the quality of life of patients with genital herpes is determined by the duration of the disease. The disease burden for more than five years leads to a change in adaptation to life with the disease, up to acceptance of obvious physical limitations.

Recently, the antiviral therapy is the most common treatment option for recurrent herpes infection. However, it should be remembered that antiviral chemotherapy drugs only suppress the active replication of the herpes virus, causing clinical remission, and do not affect the frequency of relapses. Unfortunately, frequent relapses of genital herpes cause physical and psychological discomfort, which leads to a decrease in the patient's quality of life.

CONCLUSION

Duration of recurrent genital herpes for more than five years significantly reduces the quality of life of patients, both physically and psychosocially. In the treatment of recurrent genital herpes, it is necessary to

take into account not only the effectiveness of therapeutic measures, but also psychological adaptation among women.

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CONCEPT OF THE INFLUENCE OF SARS-COV-2 VIRUSES ON CELL MALIGNANCY

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ABSTRACT — To date, in the context of the COVID 19 pandemic, there are rumors and speculations about the consequences of the infection, as well as a concern on growing cancer risk due to vaccines and vaccination. In this study we reviewed the concepts of the viral action on cancer development and analyzed the data on the possibility of the malignant effect of the SARS-CoV-2 virus on cells. Analysis of the literature data showed that SARS-CoV-2 damages cells, like other viruses, but does not lead to their mutations. There are no changes in DNA, there is only misregulation of repression and expression of the genome, a perversion of signaling intercellular interactions that disrupt the mechanisms of differentiation and specialization of cells. The need of viruses to multiply in cambial cells of tissues contributes to the induction of their proliferation and the lack of specialization. Thus, the available information on the cytopathic effects caused by viruses in cells infected with COVID-19 does not yet provide information on the malignant effect of SARS-CoV-2. Our study is aimed at collecting and analyzing data that are necessary for planning effective treatment of patients with COVID 19 and predicting outcomes in the long term after the disease.

KEYWORDS — viral concept of cancer; COVID 19; SARS-CoV-2; malignancy; apoptosis; immunity; vaccination; differentiation and specialization; genome; ischemia; hypoxia; carcinogens; cancer risk.

RELEVANCE

One of concerns COVID 19 infection impacts caused by SARS-CoV-2 is a possible induction of malignant transformation of cells. Machitani M., Yasukawa M., Nakashima J. et al. (2020) showed that the critical genetic driver of many cancers is the catalytic subunit of telomerase: human telomerase reverse transcriptase (hTERT), originally identified as an RNA-dependent DNA polymerase. However, although hTERT is a DNA polymerase, it has phylogenetic and structural similarities to viral RdRPs, and hTERT has RdRP activity, playing an important role in tumor formation. Having considered the enzymatic function of RdRP in viral proliferation and tumor development, the authors of this study came to an unexpected intersection between cancer research and RNA virus investigations [1].

The idea of microbial induction of carcinogenesis is not new; it was suggested by French scientist Bosc (1903), who found similarity in epithelial damage caused by smallpox in sheep with carcinomatous epithelioma characterized by a significant growth and which is disoriented, atypical and aggressive [2]. Bosc does not find in this, however, the final distinction between the process of parasitic epithelioma and cancer. In the latter, he believes that the corresponding parasite is limited to existence in the epithelial cell, incapable of metastasis, and concludes that viral and microbial damage belongs to the group of *sporozoan diseases*. Burye and Veno (1903) supported the concept of microbial induction of oncogenesis and suggested that *cancer microbes can migrate into tissues adjacent to the tumor* [3]. The infectious concept of carcinogenesis was supported by I.I. Mechnikov and proved by Shoup (1932-1933), Bittner (1936). And only L.A. Zilber (1936), was the first to develop the concept of viral–genetic origin of tumors and the theory of serum antibodies against the background of cancer [4]. Sadhukhan P., Ugurlu M.T., Hoque M.O. (2020) consider the problem of paramount whether survivors of COVID-19 infection are at high risk of developing cancer and whether there are any clinical features in survivors after COVID-19 that may be associated with carcinogenesis [5]. But the presence of more than 150 types of substances that are carcinogens used to obtain tumor growth in an experiment do not fit into the viral concept of cancer. At the present stage, all assumptions about the oncogenicity of SARS-COV-2 are still hypothetical, despite the fact that according to the data of the International Committee on Taxonomy of Viruses (ICTV) (2021), groups of viruses leading to the

development of tumors are indicated. The concepts of the introduction of DNA viruses into the genome of cells are untenable due to the immediate activation of the protective mechanism — apoptosis, which was shown by the morphological analysis of biopsies obtained against the background of the developed multiple organ failure as a result of infection of the organism with SARS-CoV-2. The concept of ischemia and hypoxia due to the death of only alveolocytes is not supported by the facts of the presence of shortness of breath after normalization of the parameters of the study of the respiratory system. These data are explainable in the framework of the concept of damage to SARS-COV-2 erythrocytes, as one of the main targets of viruses, at the earliest stages of erythropoiesis. In this case, the infection is accompanied by the death of cells of organs involved in erythropoiesis. Due to the fact that the mechanism of direct oncogenicity of the pathogen COVID-19, SARS-COV-2, is extremely unlikely, an analysis of the indirect effect of DNA and RNA containing viruses on cell malignancy is required. The huge number of COVID-19 cases around the world, the severity of clinical manifestations, the lack of knowledge of complications and the high genetic variability of SARS-COV-2 indicate the high relevance of studies on the condition of patients after a COVID-19 infection in the long-term period, as well as data on the consequences of vaccination. Of particular importance is the prediction of possible post-infectious carcinogenesis induced by coronavirus as a long-term complication.

MATERIAL AND METHODS

The study group included 35 patients of older age groups, of which 7 were not sick or vaccinated during the study and served as control; 12 were vaccinated a year after being discharged from the infectious diseases hospital for COVID-19, while 3 of them got sick again after contact with symptomatically infected COVID patients with carriers of the Indian delta strain; 16 were not sick and vaccinated, of which 3 were asymptomatic due to contacts 2 days before vaccination with COVID patients of SARS-COV-2 delta — a strain of etiology. The criterion for excluding patients from the study groups was the presence of cancer in the anamnesis before the disease and before vaccination. The distribution of patients is shown in Table 1.

RESULTS AND DISCUSSION

It was established that COVID-19 patients after vaccination had contact with asymptomatic infected patients who, within a week after contact, were admitted with a severe form of COVID-19 to infectious diseases hospitals. It was found that in 2 patients the clinical signs of COVID-19 were characterized as mildly symptomatic, and one developed a critical life-threatening condition that required non-invasive mechanical ventilation. Symptoms include shortness of breath, chills and fever, dry cough and weakness, fatigue, and headache. Liver damage, acute kidney injury, vasculitis and myocarditis were not observed in our studies. A concomitant disease in a seriously ill patient belonging to the older age group was arterial hypertension, obesity of the 1st level, a history of respiratory system diseases, and undergone surgical operations. Analysis of the data showed that the severity of the clinical manifestations of COVID-19 infection depends only on the initial state of the patient, his anamnesis of diseases transferred to the time of infection and against which infection with the SARS-COV-2 virus arose. Therefore, the key role in the pathogenesis of the disease is played exclusively by the interaction of the virus and the host. The high contagiousness and pathogenic properties of SARS-COV-2 viruses can cause significant tissue damage against the background of a weak, depleted and inhibited immune response, initially impaired in patients of older age groups with a burdened history. In such patients, the prognosis may not initially be entirely favorable and measures should be taken to prevent further damage to organ systems and multiple organ failure.

Current knowledge about the virological and immunological characteristics of SARS-CoV-2, the biology of the virus, the life cycle, tropism to many organs and cells, suggests how it will ultimately affect some biological and physiological functions of the host, in particular, the immune response.

Turnquist C., Ryan B.M., Horikawa I., Harris B.T., Harris C.C. (2020) suggest that analyzes of cytokine storm studies in patients during the COVID-19 pandemic will be of particular relevance to cancer research. Interleukin-6 (IL-6) has become a key component of the immune response to SARS-CoV-2, and the repurposing of anti-IL-6 therapeutic agents for COVID-19 is currently one of the main lines of research ongoing in clinical trials [6]. A framework needs to be defined to understand the role of IL-6 in the context of cancer research and the potential consequences of COVID-19. It is also necessary to use from the obtained results on immunity studies, how to induce protective reactions of cancer patients infected with SARS-CoV-2.

Van de Haar J., Hoes L.R., Coles C.E. et al. (2020) propose taking into account many common features and characteristics for prioritization to reconstruct evidence-based cancer treatment during the COVID-19 pandemic [7]. The critically necessary analysis will allow the development of an optimal anticancer

Table 1. *Distribution of vaccinated patients*

Age (years old)	Control	Vaccinated, recovered from COVID 19		Vaccinated, without a history of COVID 19	
		COVID negative (not infected after vaccination)	COVID positive (got ill after vaccination)	COVID negative	COVID positive
(20–39)		5	0	5	0
(40–59)		4	0	9	1
(60 >)		3	1	2	2
Total	7	12	1	16	3

treatment strategy that can accelerate the use of better and less toxic therapies based as a target on the immune system of patients.

Cortese M., Lee J. Y., Tserik B. et al. (2020), believes that the cause of carcinogenesis may be cell death as a consequence of inflammation and cytokine storm, showing morphological changes in organelles induced in epithelial cells of human lungs infected with SARS-CoV-2. On three-dimensional electron microscopy, ultrastructural pathological changes were characterized by reconstructions of whole cells and subcellular compartments, revealing extensive fragmentation of the Golgi complex, altered mitochondrial network and peroxisome recruitment to viral replication organelles formed by clusters of two-membrane vesicles (DMVs), as well as deep remodeling [8].

Thus, the available information on the cytopathic effects caused by viruses in cells infected with COVID-19 does not yet provide information on the malignant effect of SARS-CoV-2. At the same time, it is known that a number of pathogenic microorganisms that cause cell death do not lead to impaired restitution, and further regeneration is realized as a full-fledged one. The outcome of lung damage in patients in some cases is accompanied by complete regeneration of lung tissue, in other cases it ends in fibrosis, with filling of the defect with connective tissue, but not malignancy (Fig. 1).

Stanifer M. L., Kee C., Cortese M. et al. (2020) showed that enteric cells (hIEC), like alveolocytes, are only a productive site of replication of SARS-CoV-2, and suggested that the enteric phase of SARS-CoV-2 may be involved in the pathologies observed in patients with COVID-19 , contributing to an increase in the patient's viremia [9]. Mollica V., Rizzo A., Massari F. (2020) and Konrad H. S., Lorelei A. M., Emmanuel S. A., et al. (2020) pointed out TMPRSS2 not only as the most frequently modified gene in primary prostate cancer, but as a critical factor contributing to the infection of cells with coronaviruses, including SARS-CoV-2 [10, 11]. Hays P. (2020) believes that cancer patients are more prone to COVID-19 infection, he raises the additional question of whether COVID-19 infection induces the development of cancer [12].

CONCLUSION

Our analysis and reference literature on the mechanisms of action of SARS-CoV-2, the molecular responses that it induces upon infection in order to establish a connection between the consequences of the new coronavirus and malignancy showed that the virus is able to activate the main signaling pathways involved in aberrant cell growth, when This cytokine storm weakens the immune system's response to tumors. However, patients can develop cancer as a result not only from direct exposure to viruses, including SARS-CoV-2, but also from exposure to other carcinogenic factors present as malignant agents. We agree with Hays P. (2020) that this hypothesis should be tested in in vitro models as well as in preclinical studies. We plan to further monitor our group of patients who have been ill and vaccinated, who have

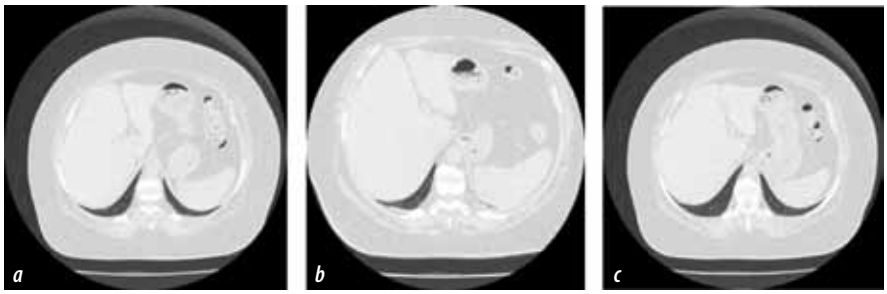

Fig. 1. *CT scan of a 67-year-old patient upon admission to the hospital with a PCR-confirmed diagnosis of COVID-19 SARS-COV-2 etiology.*
a), b) — upon admission 1.12.2020;
c) six months after recovery and discharge — 06/28/2021. Fibrous processes in lungs of patients undergoing COVID-19 are identified.

had and have not had COVID-19 for the development of cancer.

Thus, we can state that cells of various organs are the site of viral replication, leading to damage and cell death, but not to their malignancy. The recovery of patients and the restoration of lung tissue in the majority of patients indicate that the epithelium retains the ability to restitution and close defects with the performance of function. Damage to a significant part of cambial cells and ischemia, an uncontrolled inflammatory process have a detrimental effect, and the release of cytokines plays a leading role in respiratory fibrosis and multiple organ failure.

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IMPACT OF HYPOXIA AND INFECTION ON THE MORPHOLOGY OF THE THYROID GLAND

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ABSTRACT — The investigation aimed to study the microscopic features of the thyroid gland under the influence of barochamber hypoxia and staphylococcal infection in the experiment.
Materials and methods of the study. During the study 30 healthy adult male white rats weighing 180–200 g were used. Morphological features of the gland were studied by histological methods.
Results of the study. Analysis of the study results shows that morphological changes occurring in the thyroid gland cells are more pronounced in animals of the infectious group than in animals of the hypoxia group. Thus, under the influence of infection, atrophic changes prevail in the gland tissue, and under the influence of hypoxia, hyperplasia, and hypertrophy, proliferation and differentiation of gland cells are observed. This can be considered a structural reconstruction of the gland tissue and its adaptation to new conditions.

KEYWORDS — hypoxia, infection, thyroid gland, morphology, follicle.

INTRODUCTION

Nowadays, it is indisputable that social development creates a new environment with high-stress factors for individuals, as well as for society as a whole. According to Selye, *stress is a non-specific reaction of the body to any strong irritation*. The development of the body's ability to withstand stress and the general adaptation syndrome is the result of stressful influences [1]. On the other hand, stress factors play an exceptional role in the development of many pathologies in the body, including endocrine pathologies [2,3]. Thus, with chronic stress, the endocrine system, especially the thyroid gland, is more involved in the pathological process as a result of a violation of the hormonal balance, as well as intersystem connections. Stress factors such as hypoxia and infection should be particularly noted [4].

Hypoxia and inflammation are closely related pathological processes in the body at the molecular, cellular, and clinical levels, i.e. just as hypoxia can cause inflammation in the body, inflammation also causes hypoxia [5]. Thus, a violation of the body's supply of oxygen and energy leads to the formation of localized areas of hypoxia [6], and hypoxia areas — to the development of inflammatory processes. On the other hand, the inflammatory process often leads to severe hypoxia [7]. In this case, a mosaic (mixed) change in the histoarchitectonics of the thyroid gland is observed [4].

The analysis of the literature data shows that the morphofunctional rearrangement of the thyroid gland under the influence of stress factors, especially hypoxia and infection, is not sufficiently studied. Although there are currently available data on this issue in the literature, these data do not reflect many aspects of thyroid pathology in hypoxia and infection (staphylococcal peritonitis), which does not allow us to assess the role of the gland in many diseases, including endocrine pathology.

The investigation aimed to study the microscopic properties of the thyroid gland under the influence of barochamber hypoxia and staphylococcal infection in the experiment.

MATERIALS AND METHODS

During the study, 30 healthy adult male white rats weighing 180–200 g, divided into 3 groups were used. The control group (group I) did not interfere (n=10), a model of hypoxia was created on animals included in the hypoxia group (group II) (n=10), and a model of staphylococcal infection (peritonitis) was created on animals included in the infection group (group III) (n=10). For this purpose, group II animals were placed in a special pressure chamber, ventilated daily for 2 hours 5 times a week, at atmospheric pressure in the pressure chamber equal to the pressure at an altitude of 2000–3000 m above sea level, with a temperature of 19–20° C. Group III animals were infected with *S. aureus* culture by injecting 1×10^9 microbial cells/kg (per kg) in the volume of 1 ml into the peritoneal cavity. The animal studeies were conducted in compliance with the international ethical guidelines.

RESEARCH RESULTS

In histological preparations prepared from the thyroid gland of animals of the control group, the connective tissue capsule of the gland and the processes extending from the capsule and dividing the glands into separate lobules are visible. The follicles, different in shape and size, which make up the organ parenchyma are covered with a cuboid epithelium and filled with colloids (Fig. 1).

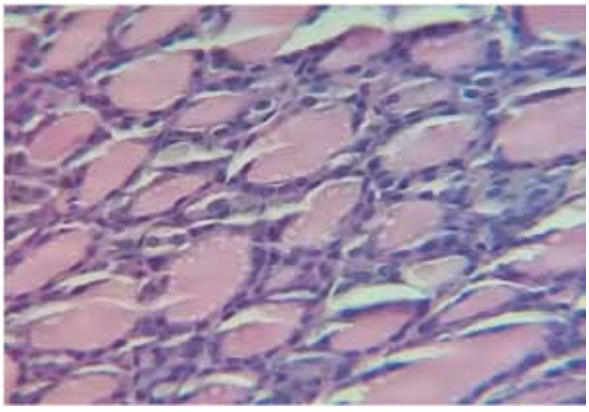

Fig. 1. *A photomicrograph of a the histological section of thyroid gland of the control rat (H&E×200)*

In histological preparations, oval-shaped follicles predominate, but there are also round follicles of small diameter. Microscopically, along with mature follicles, small immature follicles covered with prismatic epithelium from the inside and without a colloid in the cavity are also observed. Usually, the main part of the follicle consists of follicular endocrinocytes or thyrocytes. The cytoplasm of thyrocytes and the rounded nuclei located in the center of the cytoplasm are visually distinguishable.

On the 15th day of the experiment, a microscopic examination of the thyroid gland of animals of the hypoxia group showed that the cytoplasm of thyrocytes is pale, weakly eosinophilic, the nuclei are light, slightly swollen. In the center, there are hyperchromic nuclei and small vacuoles. Signs of hydropic dystrophy can be observed in thyrocytes, especially in the central zone, and the accumulation of fat droplets in some cells, but these changes are local (Fig. 2). In some areas, endothelial cell apoptosis deserves attention. Despite the structure of the thyroid stroma is normal, but weakly destroyed collagen and reticulin fibers, as well as signs of local metachromasia, a small paravasal edema are noticeable. However, under the influence of prolonged hypoxia, it is possible to see the development of fibrosis tissue along the periphery of the gland, as well as an increase in the number of fibroblasts. Thus, due to interstitial fibrosis, the thickening of the capsule of the gland and the narrowing of the follicle lumen is visible.

On the last day of the experiment (day 30), the thyroid gland of animals adapted to prolonged hypoxia acquired a normal structure and size, and the tissues that make up the structure of the gland — the parenchyma and stroma — were reconstructed. Microscopically, the capsule surrounding the gland, the lobules and septa separating them, as well as the border between the central and peripheral zones of the gland is visible. The follicles are located close to each other and have significantly increased in size. The follicular cavity is filled with a thick colloid. The cytoplasm and nuclei of thyrocytes are transparent, *light* in color. The presence of vacuoles in the cytoplasm of the cells of some follicles of the central zone indicates that fatty degeneration is not completely absorbed in these cells, but this vacuolization is very small and local (Fig. 3).

In histological preparations, the proliferation of connective tissue and fibroblasts between the follicles, the development of fibrous tissue in the interstitial space, the thickening of the gland capsule, as well as the proliferation of collagen fibers indicate the complete restoration of the glandular stroma.

The thyroid gland reacts sharply to *S.aureus* cultures injected into the peritoneal cavity. On the 15th day of the experiment, the morphological disorganization of the thyroid gland became more pronounced. The capsule of the gland is thickened, diffusely deformed in some areas, with visible erosive-scaly areas. Adhesion of neutrophilic leukocytes and mononuclear cells, macrophages, and lymphocytes with the formation of exudative inflammatory foci in the inner layer of the capsule is noted. Microscopically, the basement membrane of thyrocytes is also loosened, edematous, and relatively deformed. Most of the thyrocytes undergo dystrophic changes, mainly proteinous degeneration, some of the thyrocytes are degranulated, and some are in a state of necrobiosis and necrosis. Necrotic thyrocytes continue to desquamate into the follicle cavity, and the areas around the follicles, consisting of necrotic tissue rich with neutrophilic leukocytes and lymphocytes are visible (Fig. 4).

Atrophic processes in the parenchyma of the gland have intensified; the lobular structure is partially disturbed. The main part of the gland consists of large or cystic enlarged follicles, which are irregularly replaced by small follicles. Small atrophic follicles contain few colloids and are almost invisible. In large follicles, the vacuolized eosinophilic colloid is observed. The stroma is thickened due to the growth of connective tissue. However, the fibrous framework is deformed, diffuse stromal edema, as

well as perivascular cellular infiltration of the organ, is preserved.

At the end of the experiment — 30 days later, during the microscopic examination, the glandular parenchyma of the surviving animals (n=2) was characterized by irregularly arranged, atrophied, and deformed, small follicles with a narrow slit-like cavity devoid of colloids. There is an increase in connective tissue fibers between thyrocytes. The weakening of exudative processes leads to the activation of fibroblasts, the accumulation of macrophages and lymphocytes in the capsule and stroma of the thyroid gland, and the formation of new loose connective tissue. However, the collagen fibers that are a morphological feature of fibrillogenesis, local scarring, are mainly formed *de novo*, which is not typical for the intact gland. The follicles undergo acute hypertrophy and hyperplasia (Fig. 5).

DISCUSSION

The adaptation of the body to the effects of some endogenous and exogenous factors, including hypoxia and infection, depends on the intra-systemic restructuring of the structural elements of the thyroid gland [7], the thyroid gland plays an important role in the regulation of processes occurring under the influence of these factors. However, this regulation is complex, so it requires special research [8]. Under the influence of experimental hypoxia, the function of the thyroid gland is weakened, as well as compensatory-adaptive processes, manifested in a decrease in the height of epithelial cells of glandular tissue, changes in the diameter of follicles, changes in the ratio of epithelium and connective tissue, occur [9]. Morpho-functional changes occurring in the follicular cells of the thyroid gland in the early stages of chronic endotoxicosis — a decrease in the number of follicular thyrocytes, the development of vacuole degeneration and desquamation of some cells into the lumen of the follicle, plethora of capillaries and focal diapedesis hemorrhages in the parenchyma of the gland — are accompanied by a restoration of the activity of thyrocytes [10]. At the later stages of the experiment, the proliferation of intact follicular and interfollicular epithelium is observed, the lost parenchyma is replaced by a stroma, glandular tissue undergoes a microfollicular transformation in the form of small foci and remodeling to the macrofollicular type. With the formation of the microfollicular structure of the gland, the processes of stromal proliferation prevail, which can be considered as pathological tissue regeneration under conditions of intoxication [8]. Morphological changes occurring in thyrocytes also lead to a decrease in the concentration of hormones produced on the background of a decrease in the number of functioning thyrocytes, a weakening of the functional activity of the thyroid gland [8, 10].

According to the results of our study, under the influence of chronic hypoxia and infection, marked structural changes occur in the cells of the thyroid gland, which lead to a restructuring of the structure of the gland. But these changes are more prominent in animals of the infection group compared to animals of the hypoxia group. Thus, under the influence of infection, atrophy of both the stroma and the parenchyma of the gland gradually develops, the interaction of small and large follicles in the paren-

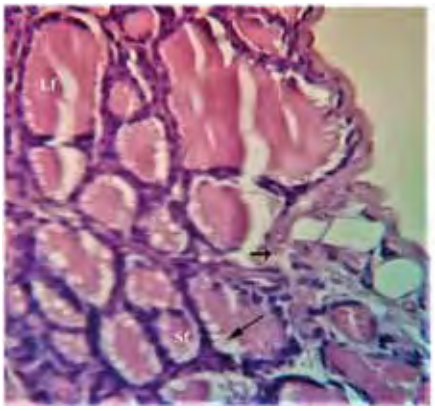

Fig. 2. *Histological section of thyroid gland of the rat showing large (Lf) and small follicles (Sf), fibrosis (thick arrow) and colloid droplets (long arrow). 15th day of the hypoxia model (H&E×200)*

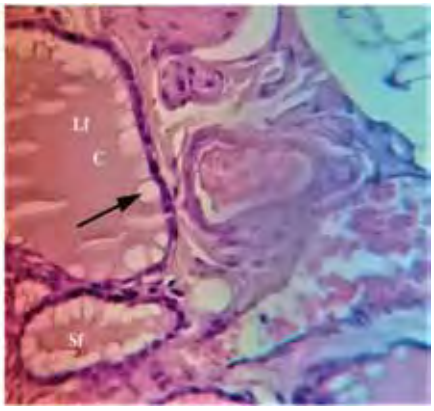

Fig. 3. *Histological section of thyroid gland of the rat showing large (Lf), and small follicles (Sf), colloid (C) and colloid droplets (arrow). 30th day of the hypoxia model (H&E×200)*

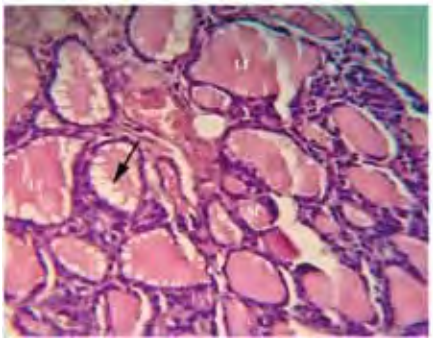

Fig. 4. *Histological section of thyroid gland of the rat showing large (Lf) and small follicles (Sf), and colloid droplets (long arrow). 15th day of the infection model (staphylococcal peritonitis) (H&E×200)*

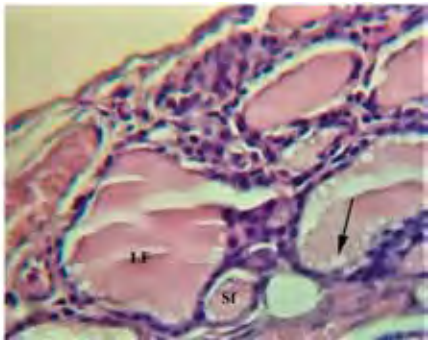

Fig. 5. *Histological section of thyroid gland of the rat showing large (Lf) and small follicle (Sf) and colloid droplets (arrow). 30th day of the infection model (staphylococcal peritonitis) (H&E×200)*

chyma is disrupted, sclerotic processes are observed in the stroma. Long-term effects of hypoxia increase the resistance of glandular tissue to hypoxia and glandular cells adapt to the hypoxic condition, the process of folliculogenesis occurs with an increase in the number of small follicles with proliferative potential and high metabolism in the parenchyma of the gland and focal growth of connective tissue in the stroma.

CONCLUSION

Thus, under the influence of infection, atrophic changes prevail in the gland tissue, and under the influence of hypoxia, hyperplasia, hypertrophy, proliferation, and differentiation of gland cells are observed. This can be considered a structural reconstruction of the gland tissue and its adaptation to new conditions.

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NEURON STRUCTURE OF THE GANGLION PLEXUSES IN THE LARGE INTESTINE

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ABSTRACT — AIM OF THE STUDY: The aim of the work: to study the syncytial connections in the ganglia of the nerve plexuses of the large intestine.
MATERIAL AND RESEARCH METHODS: The work was carried out on 30 sexually mature Wistar rats at the age of 3–4 months, having a mass of 180–320 g by the beginning of the experiment. The study used a universal method of impregnation, based on classical impregnation methods: intravascular — Rannier–Goyer and immersion — Bilshovsky–Gros. The study was conducted in 2019–2020.
RESULTS: According to our data, the large intestine has intraorgan ganglia located in the intermuscular and submucosal plexuses. The intermuscular plexus (Auerbach) of the large intestine has the form of a network with cells of various shapes and consists of nerve nodes containing cells of types I and II of Dogiel, however, the latter are numerically significantly predominant (20–25 cells or more). Syncytial connections of neurons in the autonomic ganglia of the intestine were constantly detected. These were syncytial connections of processes and bodies of two neurocytes. Protoplasmic processes of nerve cells diverge in different directions, go to meet similar branches, joining them, form a narrow or wide-looped network. Syncytial connections between the bodies of neurons and peripheral processes form closed annular anastomoses. Interfiber cytoplasmic bonds are formed by membrane syncytial fusion.
CONCLUSION. The large intestine has intraorgan ganglia located in the intermuscular and submucosal plexuses. Syncytial connections of neurons in the autonomic ganglia of the intestine were constantly detected. Interfiber cytoplasmic bonds are formed by membrane syncytial fusion.

KEYWORDS — ganglia, plexuses, large intestine, Syncytial connection, neurons, protoplasmic processes of nerve-cells, Dogiel cells.

INTRODUCTION

The study of the mechanisms for transmission of excitation in synaptic structures is one of the most urgent and rapidly developing areas in modern neurophysiology.

The human nervous system has chemical and electrical synapses (syncytial connections). The secretion of a mediator in chemical synapses is based on the process of exo-endocytosis of synaptic vesicles in the active zones of active zones [1]. Compared to chemical synapses, electrical synapses conduct nerve impulses As a rule, electrical synapses are bidirectional, that is, a nerve impulse can travel along them in both directions [2].

Electrical synapse is a place of highly specialized contacts (gap junctions) between neurons, where there is a direct flow of electrical currents from one neuron to another [3]. In gap junctions, the membranes of neighboring cells are at a distance of about 3.8 nm, while in a chemical synapse, the distance between two neurons is from 20 to 40 nm [4]. In the area of each gap contact there are many special channels that cross the membranes of both cells [5]. Ions and molecules of medium size can pass through them from one cell to another, due to which the cytoplasms of two neighboring cells are connected. A particular case of an electrical synapse is an autapse, in the formation of which an axon and a dendrite of the same neuron take part [6]. Electrical synapses (syncytial connections) are predominantly observed in the central nervous system. The basic mechanisms of this process are well understood. So, they have been studied in detail in the striatum, cerebellum and suprachiasmatic nucleus [7, 8]. However, ideas about the peculiarities of the formation of these mechanisms in various organs have significant gaps. In this respect, the ganglia of the intestinal plexuses are of particular interest.

The aim of this study

to study the syncytial connections in the ganglia of the nerve plexuses of the large intestine.

MATERIAL AND METHODS

The work was carried out on 30 sexually mature Wistar rats at the age of 3–4 months, having a mass of 180–320 g by the beginning of the experiment. Rats were chosen as a biomodel, given their physiological adequacy and simplicity of keeping. The keeping of animals and their euthanasia was carried out in accordance with the EC Directive "On the protection of animals used for experimental and scientific purposes" (86/609 CE). Universal method of impregnation was used, based on classical impregnation methods: intravascular — Rannier–Goyer and immersion — Bilshovsky–Gros [9].

The abdominal aorta was calulated to the animals under ether anesthesia and the portal vein was transected. A 5% glucose solution was first perfused through the abdominal aorta until a pure perfusate appeared in the portal vein. Then, a solution of barium hydroxide [$Ba(OH)_2$] was perfused, which is a physiological tracer passing from the bloodstream through the interstitial space into the lymphatic microvessels. Argiphilia of tissue structures is determined by the pH level of precipitation of silver hydroxide from a solution of its nitrate salt. Complete precipitation occurs at pH 11–13.Microphotography was performed from preparations of different parts of the large intestine on a Leica-DM 1000 microscope with a video system (Germany).

RESULTS

The results of the study showed that the large intestine has intraorgan ganglia located in the intermuscular and submucosal plexuses. The intermuscular plexus (Auerbach) of the large intestine has the form of a network with cells of various shapes and consists of nerve nodes containing cells of types I and II of Dogel. In the intramural nodes of the colon, there are a large number of sensitive nerve endings. At the same time, a significant number of these endings in the nodes are formed by the processes of Dogiel type 2 cells. Most of the processes of these neurons are very long, which extend beyond the node, pass as part of the internodal strands. Along the periphery of the node, these neurons have a unipolar, pseudo-unipolar, or bipolar shape, while in the center there are multipolar cells. The number of processes in these cells ranges from 2 to 6.

Syncytial connections of neurons in the autonomic ganglia of the intestine were constantly observed. These were syncytial connections of processes and bodies of two neurocytes. Based on our observations, the protoplasmic processes of nerve cells diverge in different directions, go to meet similar branches, joining them, form a narrow or wide-looped network (Fig. 1). Syncytial connections between the bodies of neurons and peripheral processes form closed annular anastomoses.

Interfiber cytoplasmic bonds are formed by membrane syncytial fusion, as reproduced in our experiments. Our preparations support the fact of independent existence of single neurons.

DISCUSSION

According to our data, modulation of synaptic transmission can occur not only at the cellular level, but also in the synaptic cleft. The synaptic cleft is filled with a loose electron-dense substance oriented parallel to the contacting surfaces.

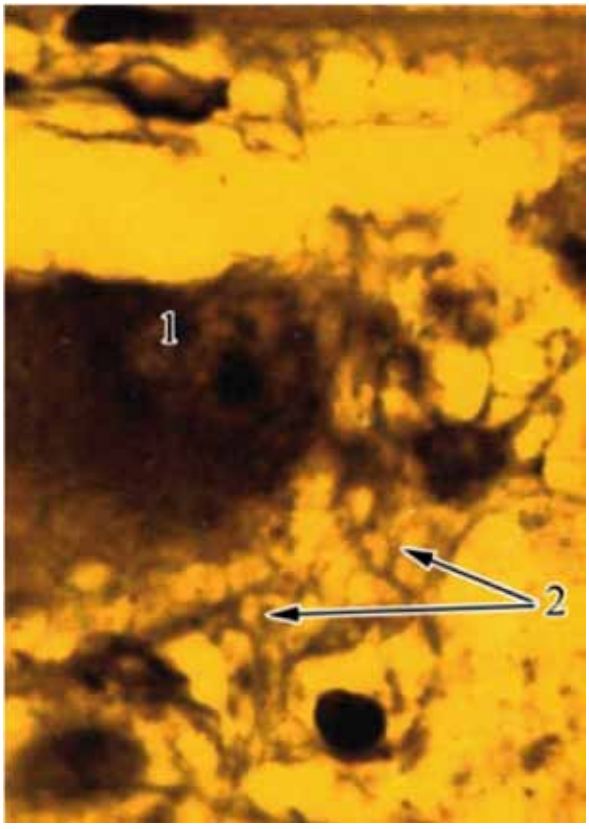

Fig. 1. *Broad-mesh network between protoplasmic processes of nerve cells. 1 — Dogel's cell of the 2nd type in the frontal plane. 2 — A wide-mesh network between the processes of nerve cells. A universal method of impregnation. Sw. ×900*

The cytoplasmic fusion of nerve processes into one neurite is a kind of *alligator's law*. Along with the great neural theory, this fact confirms and requires recognition of the validity of Dogel's reticularism and many other researchers [11]. The literature describes the reverse interaction between the postsynaptic and presynaptic parts of the membrane, carried out by various chemical agents. Currently, retrograde signals in the synapse are divided into three groups, which are related according to the principle of the release of chemical agents and their transfer from the postsynaptic part of the neuron to the presynaptic part. The first group of retrograde signals includes substances that freely penetrate the postsynaptic membrane. The second group includes substances that are secreted from the postsynaptic membrane by secretion [12].

Thus, on the set in the work by O.S. Sotnikov's question: *Is it possible with the help of light microscopy to present absolute evidence of the presence of interneuronal syncytium*, it is necessary to answer: yes, it is possible. There is no doubt that syncytial interneuronal connec-

tions make the structural organization of the nervous system more reliable. Obviously, instead of the current representation of *either — or* or synapses, or epaps, it is necessary to represent *and — and*, i.e. and synapses and epaps [12].

CONCLUSION

1. The large intestine has intraorgan ganglia located in the intermuscular and submucosal plexuses. 2. Syncytial connections of neurons in the autonomic ganglia of the intestine were constantly found. These were syncytial connections of processes and bodies of two neurocytes. Syncytial connections between the bodies of neurons and peripheral processes form closed annular anastomoses. 3. Interfiber cytoplasmic bonds are formed by membrane syncytial fusion.

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STUDY OF CANINE COLON NEOPLASMS USING TISSUE LUMINESCENCE ANALYSIS IN A HIGH-FREQUENCY ELECTROMAGNETIC FIELD

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ABSTRACT — Malignant tumors of the colon present a severe medical problem, and only their timely diagnosis can provide adequate therapy. In this work, we developed and tested a screen-ing method of endoscopic analysis of canine colon neoplasms by its luminescent glow stimulated by a high-frequency electromagnetic field. Observations were made on nine dogs with endoscopically detected tumors in the large intestine. Comparative analysis showed that benign tumor and healthy tissue areas glow at the edges in a high-frequency electromagnetic field, while malignant tumors glow over their entire site. The lumines-cence histogram of healthy tissues had a one-humped appearance, while malignant tu-mors demonstrated a double-humped character, and the brightness was higher in magni-tude. Histological analysis data confirmed the luminescence results. Research on dogs can be the basis for developing an endoscopic screening method of malignant tumors of the large intestine in humans.

KEYWORDS — neoplasm, malignant tumor, the colon, luminescence.

INTRODUCTION

Benign and malignant tumors are most common diseases of the colon, with an increasing trend [1], and only their timely diagnosis can provide adequate therapy. Endoscopic methods are widely applied for diagnosis and surgical treatment of intestinal tumors [2]. The use of fluorescence methods helps increase the informative value of endoscopic analysis [3, 4]. But the fluorescence methods have major limitations: 1) administration of fluorescence sensitizer directly into the tumor lesion; 2) creation of maximum concentration in this area (tumor/normal mucosa gradient) — a problem of selectivity; 3) phototoxicity of fluorescence sensitizer to healthy tissues outside the tumor locus [5, 6].

At present, a search for computer-aided screening technologies has been actively conducted as they can be an alternative to conventional colonoscopy after their introduction into clinical practice [7].

Luminescence in tissue can be induced by a high-frequency electromagnetic field [8]. Thus, one of the approaches in developing such a screening method can be the visualization of intestinal mucosa in a high-frequency electromagnetic field [9, 10].

Objective

The study aimed to create a screening method for diagnosing tumor-like neoplasms in the colon by visualization of luminescence in a high-frequency electromagnetic field during endoscopy.

MATERIAL AND METHODS

All experiments on animals were performed in accordance with the International Guide-lines for Biomedical Research Involving Animals, adopted by the International Council of Scientific Societies CIOMS 1985.

Endoscopic examination was performed with an Olympus colonoscope CF-H185L/I in veterinary clinic "Kovcheg" (Krasnodar, Russia) on nine 12–15 years old dogs weighing 14.7±4.2 kg and anesthetized with sodium thiopental (60 mg/kg).

In animals with tumors of the large intestine, analysis of tissue glow in a high-frequency electromagnetic field was followed by taking biopsy material for histological examination, the results of which determined the scope of surgery.

Tumor-like tissues were visualized in the high-frequency electromagnetic field for 2 seconds with a scanner KELSY-M-2011 (ELSIS company, St. Petersburg, Russia). The luminescence area was scanned, magnified by a microscope built into the scanner, recorded by a still camera (24 frames/sec; 2048×1536 resolution), and fed into a computer through a video signal digitizer. The computer program reproduced the image on the monitor screen and plotted luminance and wavelength histograms. The glow brightness was evaluated in bits, the wave-length in nanometers.

Statistical analysis of the results was carried out using "Excel" and "Statistica" programs. First, the normality of the variant distribution was determined. If the distribution law of the obtained values differed from normal, the data were presented as Me (Q1–Q3), where Me is the median, Q1 is the lower (25%), and Q3 is the upper (75%) quartiles. The nonparametric Mann-Whitney U-criterion was used for comparing two independent groups.

RESULTS AND DISCUSSION

In a healthy section of the dog's colon, a marginal glow of its wall in a high-frequency electromagnetic field was detected for all dogs. The histogram of luminescence brightness had one hump (Fig.1.1). The histogram of the luminescence wavelength reflected the beginning of the visible range (Fig. 2.1).

Polyp luminescence was also marginal; the luminescence was one-humped as well (Figure 1.2) and was more extensive than that of the healthy area (Table 1) by 37.1%. The histogram of luminescence wavelengths was at the beginning of the visible range (Figure 2.2). The min-imum limit of the luminescence range did not differ significantly from that of the healthy part of the intestine (Table 1). The maximum limit of the luminescence range was 1.7% higher than in the healthy section, and the wavelength range exceeded the range of the healthy area by 16.1%. There was no significant difference between the median wavelength ranges of the luminescence between the polyp and the healthy part of the intestine.

Thus, the differences in the glow of a dog's colon polyp from a healthy area are the stronger brightness and a more extensive wavelength range of glow in the polyp.

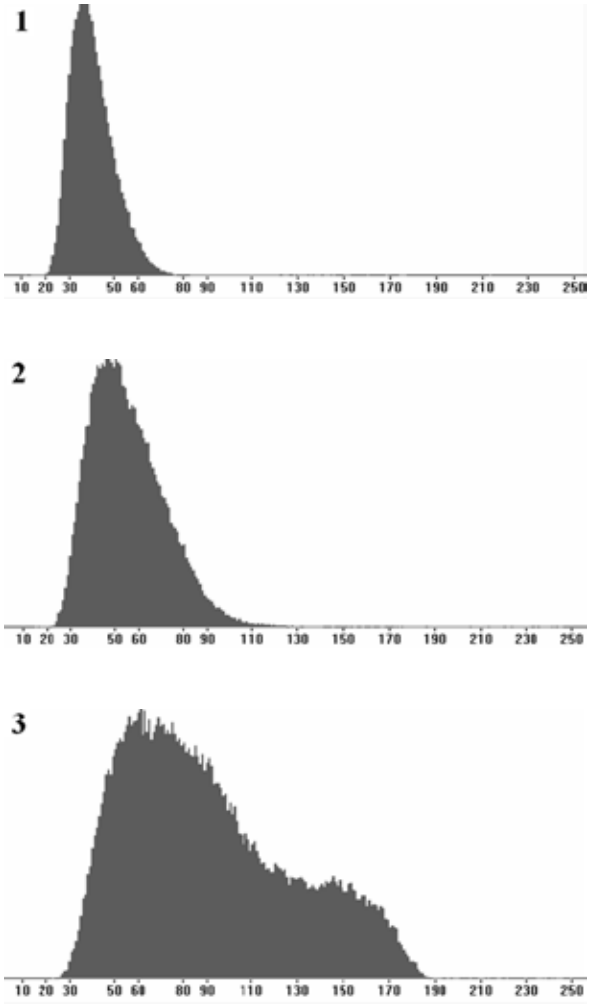

Fig. 1 *Histogram of luminescence brightness of the dog sigmoid colon (in bits). 1 — healthy section of the colon, 2 — polyp, 3 — malignant tumor*

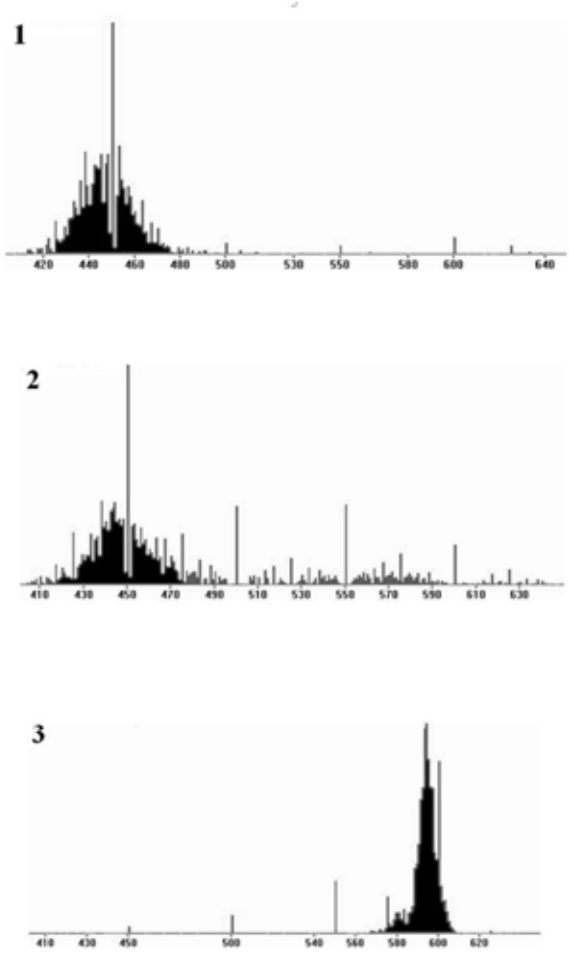

Fig. 2. *Histogram of luminescence wavelengths of the dog sigmoid colon (in nm) 1 — healthy section of the colon, 2 — polyp, 3 — malignant tumor*

Table 1. *Parameters of luminescence of different areas of the dog's sigmoid colon in the high-frequency electromagnetic field*

Glow parameters	Statistical parameters	Colon neoplasias		
		Healthy part	Polyp	Malignant tumor
		1	2	3
Luminous brightness (in bits)	Me 25% 75%	87.5 85.0 90.0	120.0 120.0 130,0 P_1 <0.01	185.0 180.0 190.0 P_2 <0.01 P_3 <0.01
Minimum limit of the luminous wavelength range (in nm)	Me 25% 75%	409.7 408.0 409.0	405.0 405.0 405.0 P_1>0.05	570.0 550.0 580.0 P_2 <0.01 P_3 <0,01
Maximum limit of the luminescence wavelength range (in nm)	Me 25% 75%	491.5 490.0 495.0	500.0 500.0 500.0 P_1 <0.01	610.0 600.0 615.0 P_2 <0.01 P_3 <0.01
Luminescence wavelength range (in nm)	Me 25% 75%	81.8 80.0 83.8	93.0 93.0 99.0 P_1 <0.01	40.0 38.0 43.0 P_2 <0.01 P_3 <0.01
Median of the wavelength range (in nm)	Me 25% 75%	450.,0 448.2 452.1	450.0 450.0 450.0 P_1>0.05	590.0 590.0 590.0 P_2 <0.01 P_3 <0.01
Diagnosis by glow parameters		Healthy part	Polyp	Malignant tumor
Diagnosis by histologic analysis		Healthy part	Polyp	Malignant tumor

P_1 — confidence interval between columns 1 and 2; P_2 — same between columns 1 and 3; P_3 — same between columns 2 and 3;

The luminescence of malignant tumor in a high-frequency electromagnetic field, along with the marginal luminescence, demonstrated an internal glow presented as a two-humped curve on the luminescence brightness histogram (Fig. 1.3). The luminescence brightness of the malignant tumor was 111.4% higher than the healthy area. The histogram of luminescence wavelength reflected the end of the visible range (Fig. 2.3). The minimum limit of the luminescence range was 39.1% higher than that of the healthy section of the intestine, and the maximum limit of the luminescence range exceeded that of the healthy part by 24.1%. At the same time, the luminescence wavelength range of the malignant tumor was less than that of the healthy area by 51.1%. The median of the luminescence wavelength range of the malig-nant tumor was 31.1% higher than that of the healthy part of the colon.

The luminescence brightness of the malignant tumor exceeded the brightness of the polyp by 54.2%. The minimum boundary of the luminescence range of malignant tumor was higher than that of the polyp by 40.2%. The maximum boundary of the luminescence range exceeded that of polyp by 22.0%. The luminescence wavelength range of cancerous tumors was less than that of the polyp by 57.9%.

CONCLUSION

The results showed that luminescence parameters of malignant tumors of the colon in the electromagnetic field in all examined animals had statistically significant differences from the luminescence parameters of healthy tissues and polyps.

Histological analysis of biopsy specimens of colon sections, polyps, and malignant tumors confirmed the diagnosis of the disease based on the luminescence parameters.

Research on dogs can be the basis for developing an endoscopic screening method of malignant tumors of the large intestine in humans.

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STRUCTURAL AND FUNCTIONAL CHANGES IN TONGUE TISSUES UNDER PER OS ADMINISTRATION OF HIGH DOSES OF LEAD ACETATE

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ABSTRACT — The main purpose of this study was to evaluate the structural transformation in rats' tongue tissue after oral administration of lead salts. Under the conditions of acute experiment during 5 days Wistar rats were orally administered the lead acetate solution in the amount of 24 mg per day. Histological studies were carried out at OSMU upon the completion of the experiment. Effect of toxic doses of lead results in symptoms of both hyperkeratosis and destruction of tongue tissue. It is assumed that the oxidative stress and tissue irritation, induced by lead intoxication, causes pathological changes in the shape, height and number of filiform papillae of the tongue. This results in hyperkeratosis, hydropic degradation of the epithelium and in the presence of an inflammatory infiltrate in the connective tissue.

KEYWORDS — lead salts, toxic doses, tongue tissue, hyperkeratosis, oxidative stress.

RELEVANCE

Lead is not a vital element. It is toxic and is a class I hazardous substance. Non-organic lead compounds disrupt metabolism and are enzyme inhibitors (like most heavy metals). One of the most treacherous consequences of impact of non-organic lead compounds is the lead's ability to replace calcium in bones and to be a permanent source of poisoning for a continuous period of time. Lately the problem of effect this metal has on human health, in particular, on oral cavity, has been a focus of attention for researchers and practitioners. Lead intoxication risk group undoubtedly includes people engaged in metal industries, but today certain diseases, which were previously diagnosed only as a consequence of impact of occupational hazards, are registered among population as well due to environmental pollution [1].

Main sources of lead intake into human body are food, water and inhaled air.

Due to man-made environmental pollution, where lead is considered a priority pollutant, information about its toxic effect on living organisms has been accumulated [2].

Degree of toxicity depends on concentration, physicochemical state and nature of lead compounds. Lead intoxication causes major changes in the nervous system, disrupts thermal regulation, blood flow and trophic processes, changes immunobiological properties of the organism and its genetic apparatus. Increased accumulation of lead in animals leads to disruption in porphyrin and heme synthesis processes, protein synthesis, in particular globulins, growing fragility and decreasing osmotic stability of cellular membranes, changes in enzyme and hormone activity. Immature nucleated red blood cells are found in blood in large numbers [4].

Lead is easily digested in gastrointestinal tract, enters almost all organs and tissues and accumulates mostly in bones and teeth, and is removed through gastrointestinal tract and salivary glands [5–6].

When assessing signs of lead intoxication, the researchers note ulcerative stomatitis, grey spots on the buccal mucosa, dense tongue plaque, increasing viscosity and volume of oral fluid sediment. Occasional symptoms are bluish or dark blue longitudinal pigmentation of the gingival margin, gingivitis, loss of pain sensation and tactile sense, taste reception, increased caries index [1]. That way lead contributes to decrease in dental health of the public. Today a number of researches offer results of histological studies of impact of lead on the oral cavity, but changes in epithelial and connective tissues of the tongue are not sufficiently studied and described.

Purpose:
to investigate structural and functional changes in tongue tissues due to toxic effects of high doses of lead acetate.

METHODS

20 Male Wistar rats weighing 260–300 g were used in the study. White rats were chosen for the study due to resemblance of structure of their oral cavity and

tongue to those of the human being. The difference is that in rats filiform papillae are more pronounced, and clavate papillae are located on the tip of the tongue, whereas in humans they are located on the tip and lateral surfaces of the tongue. Also there are differences in the oral mucosa: surface epithelium in rats is represented by multilayer keratinizing squamous epithelium everywhere, whereas in humans multilayer keratinizing squamous epithelium is located only in the area of the hard palate, gum, linea alba and filiform papillae. Blood supply, lymphatic outflow and innervation of the oral cavity in humans and in rats function similarly.

The animals were allocated to 2 groups with 10 subjects in each group: a control group and an experimental group. Rats in the control group received normal nutrition for 5 days. Rats in the experimental group received a toxic dose of lead acetate solution (24 mg daily — LD50 corresponds to 400 mg/kg) per os for 5 days. Rats were withdrawn from the experiment on day six. Decapitation was performed under ether and chloroform anaesthesia (1:1). Material used was the study was tongue segments in Bouin's fixative. Upon fixation material was washed, dehydrated and embedded in paraffin using generally accepted methods. Sections 4 μm thick were made out of the blocks and further stained with haematoxylin and eosin. After that sections were washed for 20 minutes in several changes of flowing water, dehydrated and put into a balm.

Rats were kept, looked after, fed and withdrawn from the experiment in accordance with requirements to ensuring humane treatment of animals, rules of clinical trials in the Russian Federation, approved by the Ministry of Health of the Russian Federation on December 29, 1998, provisions of the Declaration of Helsinki (2000).

RESULTS AND DISCUSSION

Fig. 1 shows the structure of filiform papillae in tongues of the rats in the control group.

Evenly spread true filiform papillae of regular size, shape and orientation are observed, covered with multilayer keratinized squamous epithelium. They are long and thread-shaped. Arched and concave sides of the papillae are fairly clearly detectible, determining posterior direction of tips of the papillae to the base of the tongue.

At the same time animals in the experimental group demonstrate loss of height of filiform papillae, increasing distance between them, destruction of tips, loss of thread-like shape and partial atrophy of the papillae attributable to oxidative stress caused by lead salts. Formation of free radicals increases whereas activity of endogenous antioxidant enzymes decreases, thus inducing cell damage and aberration of their form and atrophy.

Fig. 1. *Normal structure of filiform papillae. Haematoxylin and eosin staining, × 130*

Our findings include loss of height of filiform papillae, increasing distance between them, destruction of tips, loss of thread-like shape and partial atrophy of the papillae attributable to oxidative stress caused by lead salts. Growing formation of free radicals and declining activity of endogenous antioxidant enzymes leads to cell damage with the aberration of their form and atrophy.

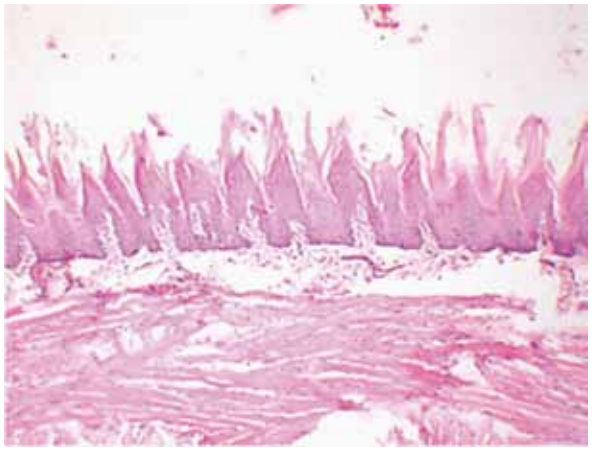

Fig. 2. *Loss of height and atrophy of filiform papillae. Hyperkeratosis. Haematoxylin and eosin staining, × 130*

Hyperkeratosis — extreme thickening of the corneous layer of epithelium — is noted. It is based on intense synthesis of keratin as a result of increasing functional cell activity of granular and spinous layers of epithelium and thickening of those layers induced by lead salts. Clinical signs are thickening of mucosa and whitening.

Inflammatory infiltrate is found in connective tissue. Cells included into inflammatory infiltrate of mu-

cosa are represented mostly by T and B lymphocytes, monocytes and dendritic cells.

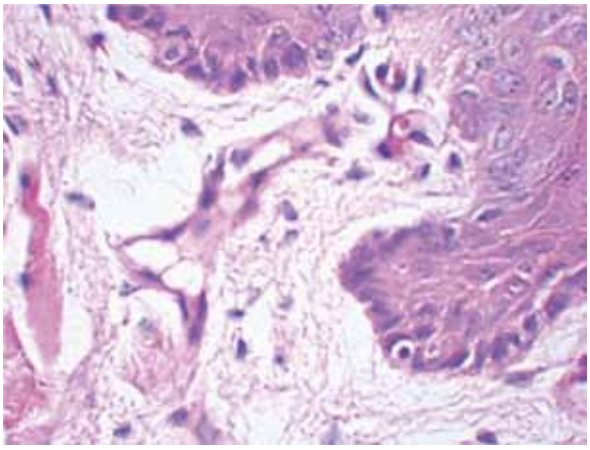

Fig. 3. *Vascular congestion in lamina propria. Moderate focal mononuclear inflammatory infiltration. Haematoxylin and eosin staining. × 900*

We observed a distinct increase in the volume of granular and spinous cell layers of epithelium. Their cytoplasm is filled with vacuoles containing transparent fluid. Nucleus in some cells is shifted to the

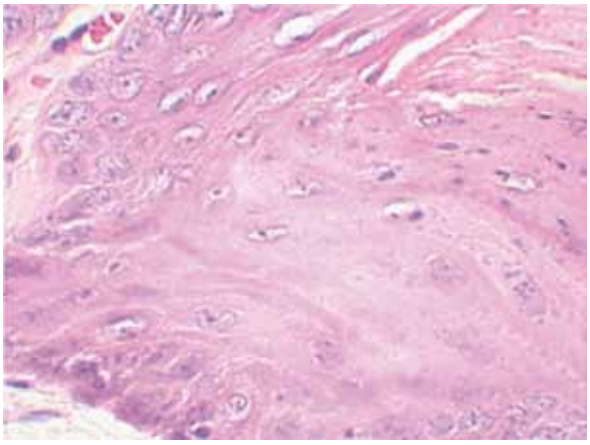

Fig. 4. *Moderate hydropic dystrophy of multilayer squamous epithelium. Haematoxylin and eosin staining, × 900*

periphery. Vacuolization or shrivelling of nuclei is also noted. Growing hydropic effect leads to breakdown of cellular ultrastructures. The mechanism of hydropic degeneration due to lead intoxication of tissues is connected with increased lipid peroxidation and decreased antioxidant capacity. This causes changes in colloid osmotic pressure in the cell, breakdown of membranes, activation of hydrolytic enzymes of lysosomes, which break intramolecular bonds during lysis with water addition [3]. Outcome of progressing hydropic degeneration turns out to be unfavourable in connection with possible total liquefactive cell necrosis.

The findings of the study have showed that highly toxic doses of lead acetate solution have a destructive effect on multilayer keratinizing squamous epithelium in the dorsum of the tongue and cause mononuclear inflammatory infiltration in connective tissue of the lamina propria of the oral mucosa. Oxidative stress and tissue irritation induced by lead intoxication lead to pathological changes in shape, height and number of filiform papillae, hyperkeratosis, hydropic dystrophy of epithelium, and development of inflammatory infiltration in connective tissue. These data corroborate pathological changes in the oral cavity caused by saturnism, which contributes to better understanding of morphofunctional pattern of lead intoxication of tissues, and may be used for prevention, diagnostics and treatment of the described abnormality.

CONCLUSION

Highly toxic doses of lead acetate solution have a destructive effect on multilayer keratinizing squamous epithelium in the dorsum of the tongue and connective tissue of the lamina propria of the oral cavity due to irritation of tissues by a toxic metal and induced oxidative stress.

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THE VARIABILITY AND THE RATIO OF THE PITUITARY GLAND AND PITUITARY FOSSA LINEAR PARAMETERS DEPENDING ON THE SKULL BASE ANGLE

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ABSTRACT — Details of the morpho- & topometric variability of the skull elements and soft tissue formations in the area of the sphenoid bone body, as well as their relationship, serve as the basis for the choice of proper surgical accesses to the respective area. The aim of this study was to identify the typical variability of morphometric parameters and the volume ratio between the pituitary gland and the Turkish saddle pituitary fossa in mature age adults. The method of computer craniometry (involving 100 MRI of people of the first and second periods of their mature age, 22–60) was employed to study the typical variability of the pituitary fossa and pituitary gland linear parameters, regardless of gender. The obtained data revealed that the regularity of morphometric variability and the volume ratio of the pituitary gland and the pituitary fossa depend on the skull base bending angle.

KEYWORDS — pituitary fossa, pituitary gland, craniotype, skull.

INTRODUCTION

The endoscopic research method, if employed in neurosurgery, will enhance significantly the visualization of hard-to-reach areas of the skull inner base, namely the Turkish saddle as well as the nearby bone and brain formations. The choice of surgical access to the respective area is based on the knowledge of the anatomical and topographic position of the skull elements and soft tissue formations and their relationship [7, 11, 28–30]. In case of the endoscopic endonasal transsphenoidal approach to pituitary neoplasm, for instance, the key anatomical points are the bottom of the Turkish saddle, the sphenoid bone platform, the sphenoid bone clivus, the carotid sulci, and the optical foramens [17].

Detailed visualization of the variability in the shape and volume of the pituitary gland, its stalk, the intersection of the optic nerves and other para-sellar structures [22–25] can be obtained through magnetic resonance imaging (MRI), which offers high image resolution.

There are a number of works available, which focus on the description of the chiasmal-sellar area anatomy [9, 10, 13, 20, 26, 27] and which reflect the dimensional features of the Turkish saddle, the shape of the pituitary fossa, along with details of the metric features and the shape of the pituitary gland as well as its topographic position in normal and pathological conditions in people of different ages, gender and race [21, 23, 24]. There have been multidirectional shaping factors identified that determine not only the structural properties of the skull, yet also its relationship with the nearest brain structures [3, 4, 6, 8, 15, 16].

However, few works contain data on the effect that the shape of the skull base has on the variability of morphotopometric parameters of the Turkish saddle and pituitary gland [14, 20], which offer little analysis of the relationship between the Turkish saddle and the pituitary gland, as well as the variability of their linear and volumetric statistical data [12]; besides, there are no comparable typical features of the said structures described. All this means that studying the typical ratio variability of the skull base elements and brain formations is an urgent issue faced by medical craniology currently, which requires detailed research.

Aim of study:

To identify the typical variability of morphometric parameters and the volume ratio of the pituitary gland and the pituitary fossa in mature age adults.

MATERIALS AND METHODS

100 archival magnetic resonance images (patients — people in their first and second periods of

mature age, 22–60) regardless of the gender factor, were studied retrospectively. The sampling included tomograms of patients featuring neither brain surgeries or pathologies, nor issues affecting the skull or brain development.

Brain images were obtained using an Aperto Hitachi magnetic resonance tomograph. Digital craniometry was done with the PACS\RIS software based on the ArchiMed system; the bone and nerve formations were evaluated on T1 and T2 weighted images in three projections.

The skull base angle relies on the craniometric points n (nasion) — s (cellare) — ba (basion), where the interval is calculated following the three sigmas (3σ) rule. Based on the basilar angle values the following 3 craniotypes were identified: mediabasilar, flexibasilar and platybasilar [2, 5].

The volume of the pituitary gland on brain tomograms was identified by measuring the following parameters in the sagittal projection: length (L) — the distance between the anterior and posterior edges of the gland; height (H) — the distance between the upper and lower edges of the pituitary gland; in the coronary projection: width (W) — the distance between the right and the left edge of the gland. The volume of the pituitary gland was calculated through the Di-Chiro–Nelson formula ($V = ½ \cdot H \cdot W \cdot L$) employed in medical radiology.

The variability of the pituitary fossa linear parameters as well as its volume was calculated through measurements in the sagittal projection: the length of the pituitary fossa (a) — the maximum distance between the anterior and posterior walls of the Turkish saddle, and the height (b) taken as the distance between the bottom of the pituitary fossa and the plane running from the saddle tubercle to its back; in the coronary projection: width (c) — the distance between the medial edges of the carotid sulci (10). The volume of the Turkish saddle was calculated relying on the ellipsoid formula ($V = ¾ \cdot \pi \cdot a \cdot b \cdot c$), where a, b, and c are the lengths of the semi-axes.

The obtained data distribution was checked via the Shapiro–Wilk and Kolmogorov–Smirnov criteria relying on the Lilliefors correction. Since the analyzed distribution did not differ from the normal one, the statistically significant differences between the average values were evaluated based on the Student's criterion, whereas the difference was considered statistically significant at $P<0.05$. The coefficient of variation (Cv%) was estimated following Lakin G. F. (1980), where at the variation coefficient of up to 10%, the variability was interpreted as weak; at 11–25% — average, and in the event the said value exceeded 25% the variability was considered strong.

RESULTS

In case of the flexibasilar craniotype, the pituitary gland length is 3.8 mm shorter (7.7±0.2 mm) than its width (11.5±0.3 mm) while exceeds its height by 2.1 mm (5.6±0.2 mm, $P<0.05$); the volume of the pituitary gland is 254.1±16.1 mm. The length of the Turkish saddle pituitary fossa is 3.1 mm shorter (10.1±0.2 mm) than its width (13.2±0.3 mm) and 1.1 mm longer than its height (9.0±0.2 mm, $P<0.05$), whereas the volume of the pituitary fossa is 630.9±21.1 mm. The length of the pituitary gland is 2.4 mm shorter (7.7±0.2 mm) than the length (10.1±0.2 mm) of the pituitary fossa, while the width of the pituitary gland is 1.7 mm shorter (11.5±0.3 mm) than the width (13.2±0.3 mm) of the pituitary fossa, and the height of the pituitary gland is 3.4 mm shorter (5.6±0.2 mm) than the height (9.0±0.2 mm) of the pituitary fossa ($P<0.05$). The pituitary gland was found to take up 40.2 % of the pituitary fossa volume. All the parameters in question feature an average degree of variability of the characteristic.

As for the mediobasilar craniotype, the length of the pituitary gland is by 4.4 mm shorter (8.6±0.2 mm) than its width (13.0±0.3 mm) while exceeds the height by 2.7 mm (5.9±0.2 mm, $P<0.05$); the volume of the pituitary gland is 334.3±19.8 mm. The length of the pituitary fossa of the Turkish saddle is 3.3 mm shorter (10.2±0.2 mm) than its width (13.5±0.3 mm), and by 2.0 mm exceeds its height (8.2±0.2 mm, $P<0.05$), whereas the volume of the pituitary fossa is 588.9±21.8 mm. The length of the pituitary gland is 1.6 mm shorter (8.6±0.2 mm) than the length (10.2±0.2 mm) of the pituitary fossa, while the width of the pituitary gland is 0.5 mm shorter (13.0±0.3 mm) than the width (13.5±0.3 mm) of the pituitary fossa, and the height of the pituitary gland is 2.3 mm shorter (5.9±0.2 mm) than the height (8.2±0.2 mm) of the pituitary fossa ($P<0.05$). The pituitary gland was observed to occupy 56.6 % of the pituitary fossa volume. All the parameters studied were found to reveal an average degree of the variability of the characteristic.

As far as the platybasilar craniotype is concerned, the pituitary gland length is 3.7 mm shorter (9.2±0.2 mm) than its width (12.9±0.4 mm) at the same time being 3.1 mm longer than its height (6.1±0.2 mm, $P<0.05$); the pituitary gland volume is 368.1±26.7 mm. The length of the pituitary fossa is 4.2 mm shorter (10.8±0.2 mm) than its width (15.0±0.4 mm) and by 2.9 mm exceeds its height (7.9±0.2 mm, $P<0.05$). The volume of the pituitary fossa is 673.8±32.3 mm. The length of the pituitary gland is 1.6 mm shorter (9.2±0.2 mm) than the length (10.8±0.2 mm) of the pituitary fossa, the width of the pituitary gland is 2.1 mm shorter (12.9±0.4 mm) than

the width (15.0±0.4 mm) of the pituitary fossa and the height of the pituitary gland is 1.8 mm shorter (6.1±0.2 mm) than the height (7.9±0.2 mm) of the pituitary fossa (P<0.05). The pituitary gland proved to occupy 54.6% of the pituitary fossa volume. The results obtained showed an average degree of the characteristic's variability.

COMPARATIVE ANALYSIS AMONG THE SKULL BASE TYPES

In the flexibasilar craniotype, the length of the pituitary gland is 0.9 mm shorter than in case of the mediobasilar and 1.5 mm shorter compared to the platybasilar type (P<0.05). The height of the pituitary gland features no significant differences in craniotypes. The width of the pituitary gland in the platy- and the mediobasilar types shows similar values and exceeds by 1.4 mm (P<0.05) the same dimension in the flexibasilar type. The pituitary gland volume was found to be the smallest for the flexibasilar craniotype, if compared to the mediobasilar one, where the volume is larger by 80.2 mm^3, and in case of the platybasilar type – by 114 mm^3.

The length of the pituitary fossa in case of the platybasilar craniotype exceeds that of the flexibasilar type by 0.7 mm, and by 0.6 mm — of the mediobasilar (P<0.05), while this parameter does not statistically differ between the flexi- and mediobasilar types. The height of the pituitary fossa features a significant prevalence in the flexibasilar craniotype if compared to the mediobasilar type — by 0.8 mm, and by 1.1 mm — compared to the platybasilar, while this parameter shows no statistically significant difference for the medio- and platybasilar types. The width of the pituitary fossa was the biggest in the platybasilar craniotype, exceeding by 1.8 mm that of the flexibasilar, and 1.5 mm — of the mediobasilar types (P<0.05). In the platybasilar type, the volume of the pituitary fossa is 83.4 mm^3 larger than in the mediobasilar type (P<0.05), with no statistical difference observed between the volume pertaining to the other types of the skull base.

A comparative analysis of the ratio between the pituitary fossa volume and the volume of the pituitary gland showed that the largest volume of the pituitary fossa is occupied by the pituitary gland in case of the mediobasilar and platybasilar craniotypes (56.6% and 54.6%, respectively), thus exceeding — by 16.3% and 14.3% — similar volume indicators in case of the flexibasilar type (40.3%).

DISCUSSION

The morphology of the sphenoid bone Turkish saddle is of applied value for clinical anatomy, since its shape and size allow making judgments of the pituitary gland status [13]. Our study revealed not only the typical morphometric variability of the pituitary fossa and of the pituitary gland, yet also of their volume ratio. The largest values of the length of both the pituitary fossa and the pituitary gland, for instance, are to be observed in the platybasilar craniotype, the smallest being found in the flexi- and mediobasilar types. Some authors note that the shape and size of the Turkish saddle depend on the height of the skull and its base length [18], while others deny the dependence between the shape, the Turkish saddle and the shape of the skull [9, 10].

The data on the age and sex variability of the pituitary gland linear parameters are scarce and contradictory [21–25], while they do not reflect any relationship with the arch shape and the skull base. The average values of the pituitary gland size obtained through this study fall within the range of the values presented by the said authors without taking into account the craniotype. We also identified the typical variability of the pituitary gland width and length with the predominance of these parameters in the platybasilar craniotype, and did not detect the dependence of its height on the type of skull base. The volume ratio of the studied structures showed that more than 50% of the pituitary fossa volume is occupied by the pituitary gland in the medio- and platybasilar types, and only 40.3% — in case of the flexibasilar type, whereas the results of such a ratio without taking into account the typical variability [12] ranged from 68% to 100%, the average value being 86%. Given the lack of research and publications, it appears important to follow studying the features of the typical morphometric variability of soft-tissue brain and skull structures, employing advanced research methods.

CONCLUSION

Hence, the regularity of morphometric variability and the volume ratio of the pituitary gland and the pituitary fossa depend on the bending angle of the skull base. All the craniotypes featured the width of the pituitary gland and pituitary fossa prevailing over their length and height. The extreme types reveal variability of the volumetric and linear characteristics of the elements under study: the platybasilar craniotype has the highest values of the linear parameters of the pituitary gland, the length and width of the pituitary fossa, as well as the volume ratio between the gland and the pituitary fossa; the flexibasilar type was found to have the lowest values of the same parameters and the volume ratio of these elements, yet with the height of the pituitary fossa dominating if compared to other types of the skull base. As for the mediobasilar craniotype, the linear parameters fall within the range of values of

the extreme types of the skull base, while the volume ratio of the pituitary gland and of the pituitary fossa correspond to the platybasilar type.

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THERAPEUTIC EFFECT OF SINGLET OXYGEN ADMINISTRATION ON CRYSTALLIZATION OF RATS' BLOOD SERUM AT THERMAL TRAUMA

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ABSTRACT — Biological and therapeutic effects of singlet oxygen have not been investigated yet. The aim of this paper is to estimate the influence of a short course (10 days) of singlet oxygen inhalations on crystallogenic properties of rats' blood serum. The experiment was performed on 30 male Wistar rats, randomly divided into three equal groups. The first group was intact. The animals of the 2nd (control) and 3rd (test) groups under combined anesthesia were subjected to thermal trauma. Starting from the day following the injury, the rats of the test group inhaled daily the air flow from a singlet oxygen generator during 10 days. Blood samples were obtained from the rats of all groups. Dried samples were evaluated visually for crystallizability, structure index, facia destruction degree, and marginal zone clarity, using respective scales. It is stated that singlet oxygen inhalations facilitate the elimination of negative transformations in blood crystallization induced by thermal trauma. It indicates the positive rehabilitation potential.

KEYWORDS — singlet oxygen, inhalations, crystallization, blood serum.

INTRODUCTION

It is known that inhalations of a gas stream of singlet oxygen are perceived by a number of researchers as a means of rehabilitation that improves adaptive capabilities of the body in sports medicine and in the treatment of a number of therapeutic diseases [1, 2]. At the same time, the systemic and molecular mechanisms of its action are still poorly studied [2, 3]. In this regard, we conduct systematic studies aimed at verifying and deciphering the effects and mechanisms of the influence of singlet oxygen on biological systems in physiological and pathological conditions. Thus, it was shown on human blood samples that the treatment with singlet oxygen stimulates antioxidant systems, the energy metabolism of red blood cells and optimizes the parameters of acid-base equilibrium [1, 4]. In healthy animals, a ten-day course of singlet oxygen inhalations also caused an antioxidant effect and activation of energy metabolism in the blood and tissues [5].

The aim of the study was to study the dynamics of the crystallogenic properties of rat blood serum during a course of singlet oxygen inhalation in the post-burn period.

MATERIAL AND METHODS

The experiment was performed on 30 male Wistar rats (body weight – 200–250 g, 2–2,5 month old), randomly divided into three equal groups. The first group (n=10) was intact, the animals did not undergo any manipulations, but only once received blood from the hyoid vein. The animals of the 2nd (control) and 3 (main) groups under combined anesthesia ("Zoletil" + "Xylavet") were subjected to thermal trauma according to our method, standard local treatment was carried out [6]. Starting from the day following the injury, the rats of the main group were inhaled daily for 10 days with an air flow coming from a singlet oxygen generator. To create a gas mixture including singlet oxygen, the Airnergy Professional Plus device (Germany) was used [3]. The duration of each procedure was 10 min. The generator power is 100%. The next day after the completion of the full course of inhalations, blood samples were obtained from the sublingual vein in rats of this group for research. At the same time, blood was taken from animals of group 2. All blood samples were centrifuged at 1500 rpm for 15 min. Then, 100 mcl. of blood serum was applied to a slide and were prepared with the crystalloscopy method [6, 8]. The dried slides (facias) were evaluated morphologically and visuametrically [6]. The main indicators evaluated on a point scale were crystallizability (the density of crystalline elements), the structure index (characterizes the complexity of structure), the degree of destruction of the facias (indicator of the correctness of structures formation) and the severity of the marginal zone of the facia.

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

Statistical processing of the results was performed using Statistica 6.1 program.

RESULTS

A comparison of the crystalloscopic images of the blood serum of rats of the intact and control groups allowed us to confirm the previously shown patterns of transformation of the dehydration structuring of the biofluid associated with thermal trauma [6]. They are manifested in a pronounced inhibition of the crystallogenic activity of the biological medium, a simplification of the composition of elements and a sharp increase in the number and degree of destruction of the destroyed elements, as evidenced by shifts in the levels of crystallizability, the structural index and the degree of destruction of the facies, respectively. At the same time, the size of the marginal zone of the micropreparation in burned animals was significantly reduced, which is realized in a change in the corresponding morphometric indicator.

These trends of changes are realized in shifts of crystallogenic activity of animal blood serum (Fig. 1). Thus, a pronounced inhibition of the crystallization of biological fluid is indicated by a sharp drop in the level of crystallizability and the structural index in rats with thermal trauma compared with intact animals ($p<0.05$ for both indicators). Against this background, the processes of structure construction in drying samples of the biological environment are disrupted, which is clearly evidenced by a significant increase in the degree of destruction of the crystallogenesis facies ($p<0.05$ in relation to healthy rats) — the main criterion of *correctness* [6]. Hypoproteinemia and dysproteinemia formed in burned animals are reflected in a decrease in the relative width of the marginal zone ($p<0.05$) containing proteins of the native structure and estimated by the level of the short-circuit parameter [9, 10, 13–15]. Conducting a course of inhalation therapy significantly reduces the severity of pathological shifts in the crystallogenic properties of rat blood serum (Fig. 1). It should be emphasized that the studied effect contributed to the normalization of all the indicators of the crystalloscopic test, and they statistically significantly differed both from the level characteristic of rats with thermal trauma and from the level of healthy animals ($p<0.05$ for all cases).

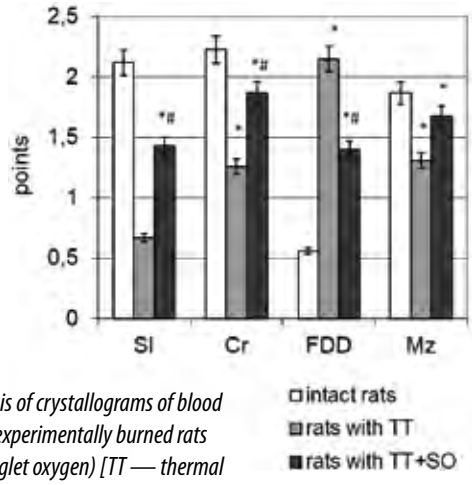

Fig. 1. *Criterial analysis of crystallograms of blood plasma in intact and experimentally burned rats (with and without singlet oxygen) [TT — thermal trauma, SO — singlet oxygen; SI — structure imdex, Cr — crystallizability, FDD — facia destruction degree, Mz — clearity of marginal zone; "*" — p<0,05 to intact rats, "#" — p<0,05 to burned rats]*

CONCLUSION

In general, it was found that the inhalation of singlet oxygen in animals with severe thermal trauma contributes to the partial normalization of the crystallogenic activity of blood serum, which positively characterizes its rehabilitation potential.

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EFFECT OF MELANOCORTINS AND EXPERIMENTAL SOCIAL STRESS ON THE LEVEL OF CASPASE-3 AND CASPASE-8

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ABSTRACT — At present the study of stress-generating effects and key mechanisms of regulation of apoptosis remain in the focus of attention of researchers. Stress causes dysfunctions of many body systems which lead to disruption of homeostasis and molecular cell mechanisms of programmed cell death. In this context, the problem of pharmacological regulation and correction of these disorders poses an urgent problem. Neuropeptide compounds such as melanocortins are interesting in this direction as regulators and correctors. Under conditions of experimental social stress activation of apoptotic processes was established due to an increase in the level of caspase-3 and caspase-8. The studied neuropeptides Semax (ACTH (4-7)-Pro-Gly-Pro) and ACTH (6-9)-Pro-Gly-Pro exhibit antiapoptotic effects under conditions of induced social stress in rats. This action of neuropeptides is manifested by a decrease in the activity of the initiating and effector caspases in the blood serum of experimental animals.

KEYWORDS — experimental social stress, neuropeptides, melanocortins, Semax, ACTH(4-7)-Pro-Gly-Pro, ACTH(6-9)-Pro-Gly-Pro, apoptosis, caspases.

INTRODUCTION

Nowadays the study of the impact of stress, including social stress, on various pathological processes in the body is an actual problem. It has been proven that stress causes dysfunction of many body systems [5, 11]. Numerous studies have been carried out confirming the fact that the influence of stress factors can lead to the development of endocrine, immune, antioxidant, metabolic and other types of disorders leading to disruption of homeostasis, which in turn in its turn can damage molecular-cell apoptotic mechanisms [3, 4, 8]. In recent years the assessment of apoptotic processes is often carried out by determining the activity of caspases, both initiator and effector. Caspases are able to activate each other, forming a caspase cascade. In the works of modern researchers much attention is paid to the study of caspase-3 and caspase-8, which makes it possible to determine the receptor pathway for the initiation of programmed cell death [1, 2]. It has been established that the process of initiation of effector caspase-3 by caspase-8 is characteristic of the development of apoptosis in lymphoid and endothelial cells, which in turn contributes to the development of immune dysfunction, as well as pathology of the cardiovascular, urinary and other systems. It should be noted that when the body is exposed to stress factors, activation of apoptotic processes is observed, including caspase-3-dependent apoptosis [6]. A group of scientists have proven that, along with caspase-3, caspase-8 plays one of the main roles in the realization of pathological conditions observed under the influence of chronic stress [1, 7]. In this connection, the problem of pharmacological regulation and correction of these changes is becomes ever more urgent. Neuropeptide compounds that play the role of regulatory molecules that are produced by almost all body systems, which determines their broad pharmacological activity, are of great interest in this direction. It has been established that neuropeptides are capable of exerting metabolic and antioxidant activity, as well as participating in the processes of induction of various trophic factors and cytokines, regulation of apoptotic processes [8, 10, 11].

Melanocortins are of particular interest as promising remedies for the correction of disorders in the mechanisms of apoptosis: Semax (ACTH(4-7)-Pro-Gly-Pro) and ACTH(6-9)-Pro-Gly-Pro, synthesized at the Institute of Molecular Genetics of the National Research Center Kurchatov Institute.

The aim of research:
to study the effect of melanocortins on the level of initiating and effector caspases in the blood serum of white rats under conditions of experimental social stress.

MATERIAL AND METHODS

The study was carried out on 70 male white rats aged six months. All manipulations with laboratory animals were performed in accordance with the requirements of international and national regulations on the protection of animals used for scientific purposes.

When modeling *social* stress, we adhered to the conditions when animals lived in conditions of sensory contact with the subsequent formation of an aggressive and submissive type of behavior. In order to form *social* stress, the animals were separated in pairs by a transparent partition in the cage, which provided sensory contact in the absence of physical contact. The partition was removed for 10 minutes every day and the inter-male confrontations were observed. As a result, groups of rats with aggressive (aggressor) and submissive (victim) types of behavior were formed. Aggressiveness of rats was assessed by the presence of attack and vertical and lateral stands, and submissiveness by the presence of immobility, sniffing, autogrooming, and vertical *protective* stands.

After stressing the rats were divided into groups: intact males (control); animals exposed to *social* stress for 20 days (stress); and experimental groups which received intraperitoneally Semax and ACTH(6-9)-Pro-Gly-Pro at a dose of 100 μg/kg/day for 20 days starting from the 1st day of stress exposure.

The effect of neuropeptides on the activity of apoptosis processes was assessed by determining the level of initiating and effector caspases (caspase-8 and caspase-3) (ELISA Kit for Caspase-8 and ELISA Kit for Caspase-3; USA) in the blood serum of white rats by enzyme immunoassay.

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

RESULTS

The results reflecting the effect of melanocortins on the activity of apoptosis processes were assessed by determining the level of caspase-3 and caspase-8 in the blood serum of white rats under conditions of experimental social stress, are presented in the Table 1.

In groups of experimental animals with an aggressive type of behavior, the following changes were noted: the level of caspase-3 in the stress group increased 1.8 (p1<0.05) times in comparison with the control group; compounds ACTH(4-7)-Pro-Gly-Pro and ACTH(6-9)-Pro-Gly-Pro contributed to a decrease in the level of the studied marker in 1.4 (p_2<0.05) and 1.2 (p_2<0,05) times, respectively, in comparison with the *social* stress group. A change in the level of caspase-8 was noted: in the group of *social* stress, an increase of 2.6 (p_1<0.05) times was noted in relation to the control group; against the background of the introduction of ACTH(4-7)-Pro-Gly-Pro and ACTH(6-9)-Pro-Gly-Pro, a decrease in the level of the indicator in 2 (p_2<0.05) and 1.1 (p_2>0,05) times in comparison with the group of stressed animals respectively.

In groups of animals with a submissive type of behavior when determining the level of caspase-3, the following changes were noted: in rats of the *social* stress group, this indicator increased 1.6 (p_1<0.05) times in relation to the control group; under the conditions of administration of the test compounds a tendency towards a decrease in this marker was observed; the level of caspase-8 increased 2.4 (p_1<0.05) times in relation to intact animals; the administration of the compounds ACTH(4-7)-Pro-Gly-Pro and ACTH(6-9)Pro-Gly-Pro contributed to a decrease in the level of the studied marker by an average of 1.3 times (p_2>0.05) compared to stressed rats.

According to the results of the study, it was found that under conditions of experimentally social stress, an increase in apoptotic processes was observed accompanied by an increase in the level of caspase-3 and caspase-8 in the blood serum of white rats. The administration of Semax (ACTH (4-7)-Pro-Gly-Pro) and ACTH (6-9)-Pro-Gly-Pro against the background of stress contributed to a decrease in the level of the studied parameters, which is most likely associated with the presence of antiapoptotic action in melanocortins due to inhibition of the caspase-dependent cascade of apoptosis reactions.

This pathway, along with caspases, is realized with the participation of cell death receptors, which include tumor necrosis factor. Previously, the presence of antioxidant action and the ability of melanocortins to influence the level of pro- and anti-inflammatory cytokines have been proven. It has been established that Semax (ACTH(4-7)-Pro-Gly-Pro) and ACTH(6-9)-Pro-Gly-Pro under conditions of *social* stress cause an inhibition of free radical oxidation processes and reduce the concentration of pro-inflammatory cytokines such as IL-1β, IL-6 and TNF-α [9, 10].

CONCLUSION

Thus, effector caspase-3 and initiating caspase-8 are actively studied as targets for various pathological conditions, in which the processes of programmed cell death are triggered. Expression of caspases-3 and -8 is an indicator of the cytotoxicity of an apoptotic stimulus, which makes these markers an important part of research on apoptotic processes. These molecules-markers of the external and internal pathways of apoptosis can serve as targets for the action of stress-protective drugs of neuropeptide structure. The study established the presence of Semax

Table 1. *The level of caspase-3 and caspase-8 in blood serum of white rats under conditions of experimental social stress and administration of melanocortins*

Groups of experimental animals	Markers of apoptosis	
	Caspase-3 (pg / ml)	Caspase-8 (pg / ml)
Control	17,41±1,22	2,33±0,91
Animals with an aggressive type of behavior		
Experimental social stress	30,62±4,23*	6,14±1,21*
Experimental social stress + ACTH(4-7)-Pro-Gly-Pro	21,49±1,23#	3,08±0,91#
Experimental social stress + ACTH(6-9)-Pro-Gly-Pro	24,84±2,11	5,15±1,20
Animals with submissive type of behavior		
Experimental social stress	27,83±3,81*	5,64±0,87*
Experimental social stress + ACTH(4-7)-Pro-Gly-Pro	23,74±1,42	4,26±1,22
Experimental social stress + ACTH(6-9)-Pro-Gly-Pro	24,30±2,13	4,58±0,98

Note: ** — p1 <0.05; ** — p1 <0.01; *** — p1 <0.001 — relative to the control; # — p2 <0.05; ## — p2 <0.01; ### — p2 <0.001 — relative to stressed animals (Student's t-test)*

(ACTH(4-7)-Pro-Gly-Pro) and ACTH(6-9)-Pro-Gly-Pro under stressful effects of anti-apoptotic action due to inhibition of the caspase-dependent cascade of apoptosis reactions.

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THE DYNAMICS OF LUNG HISTOPATHOLOGY IN ACUTE BACLOFEN POISONING

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ABSTRACT — Baclofen (sold under the name Baclosan©, Lioresal©) is a muscle relaxant. This drug is chemically different from other muscle relaxants. Due to its pronounced psychotropic effect the drug is often a subject of abuse especially among young people. The article deals with the dynamics of lung histopathology in acute baclofen poisoning. Experimental studies were performed on 15 Wistar rats. The animals were divided into 3 groups (the controls and two experimental groups). The controls included 5 intact rats. Each experimental group included 5 animals. Both groups were treated with baclofen at a dosage of 85 mg/kg. The duration of the experiment was 3 and 24 hours, respectively. We revealed a complex of pathological changes in the lungs of the rats. There were circulatory disorders in all the elements of the microcirculatory bed, areas of emphysema, atelectasis and dystelectasis, WBC infiltration of intralveolar septa and their thickening due to edema. The changes were even more severe 24 hours after the drug administration. These results along with the results of chemical analysis will be useful in establishing the fact of baclofen intoxication and the exact moment of the intoxication.

KEYWORDS — Baclofen, poisoning, lungs, lung histopathology.

INTRODUCTION

Baclofen (sold under the name Baclosan©, Lioresal©) is a muscle relaxant. This drug is chemically different from other muscle relaxants and represents a beta-p-chlorophenyl derivative of an inhibitory neurotransmitter GABA (gammaaminobutyric acid) [1, 2].

Baclofen is presented in oral and intrathecal forms and is widely used in clinical practice [3]. Oral Baclofen is indicated for patients with multiple sclerosis, severe muscle spasticity, and various spinal cord diseases (including injuries, acute circulatory disorders, tumors and infections). The drug has been shown to be effective in the treatment of patients with alcoholism [4–6] and cerebral palsy [7–8]. The mechanism of baclofen action still remains unclear. The drug has been shown to inhibit monosynaptic and polysynaptic reflexes, reduce excitability of gamma-motoneurons, which underlies its myorelaxant effect [8].

Adverse effects when using baclofen may include drowsiness, headache, dizziness, weakness, fatigue, trouble sleeping, nausea and vomiting, urinary retention, constipation.

During treatment with baclofen, an increase in body weight may be observed. Baclofen has a hepatotoxic effect under prolonged use [1, 2]. Acute baclofen intoxication can result from an accidental overdose, criminal acts or suicidal behavior [9]. There is no specific antidote in acute baclofen poisoning. Symptomatic therapy (such as cardiovascular support, mechanical ventilation if needed) is recommended in such cases. Baclofen has a pronounced psychoactive effect and can be a subject to abuse by mostly young people [10, 11].

One of target organs in baclofen poisoning are lungs. As far as we know, no previous research has investigated lung histopathology in acute baclofen intoxication.

MATERIALS AND METHODS

The experiment was performed on 15 male Wistar rats weighing 290–350 g and aged 20 weeks.

The animals were kept and handled in accordance with the European Convention for the Protection of Vertebrate Animals used for experiments or other scientific purposes (Strasburg, March 18, 1986). Baclofen was administered under general anesthesia (chloralose) through a gastric tube.

The animals were divided into the following groups:

- control (n=5) which included animals that received normal saline 10 ml/kg;
- group 1 (n=5) comprising animals receiving baclofen 85 mg/kg in normal saline solution with the experiment duration of 3 hours;
- group 2 (n=5) which consisted of animals receiving baclofen 85 mg/kg in normal saline solution with the experiment duration of 24 hours.

After the experiment, the animals were euthanized by an overdose of anesthesia. The lungs were

fixated in 10% neutral formalin and embedded in paraffin. Histological sections of 5 μm thickness were prepared, placed on slides and stained with hematoxylin and eosin. Then the sections were examined by light microscopy using a Nikon Eclipse E-400 microscope with a video system based on a Watec 221S camera (Japan) at ×400 magnification.

The following signs were assessed: circulatory disorders (capillary and venous plethora, hemorrhages in interalveolar septa, hemorrhages in alveoli, sludge), atelectasis (complete and partial), emphysema, cellular response (increased WBCs in interalveolar septa), epithelial desquamation in the bronchial lumen.

Fisher criterion was used to assess the significance of a particular histological sign. The sign was considered significant if it was absent in one group and appeared in 4 or 5 cases in another group.

RESULTS

No pathologic changes were observed in the lungs of the controls.

Three hours after baclofen administration (85 mg/kg), we revealed circulatory disturbances (venous and capillary plethora, sludge), areas of emphysema, atelectasis (complete and incomplete), and cellular response (WBC infiltration of interalveolar septa). We also revealed areas of thickening of interalveolar septa due to edema. All the signs were considered significant.

24 hours after baclofen administration we also observed emphysema in the lungs of experimental animals. The presence of dystelectases, areas of thickening of the interalveolar septa (due to edema) was also significant, as well as circulatory disorders (capillary, venous plethora, a significant number of hemorrhages in the interalveolar septa). We revealed hemorrhages in the alveoli, which was not observed 3 hours after baclofen administration.

DISCUSSION

According to the literature [1, 2] baclofen has no direct toxic effect on the bronchi and lungs. However, the drug increases presynaptic blockade of nerve impulses originating in the spinal cord, which inhibits their transmission. The tone of the muscles (including the intercostal ones) decreases. Their excessive relaxation can cause breathing difficulties followed by hypoxia. GABA A receptor agonists are known to cause contraction of bronchial and bronchiolar smooth muscles accompanied by spasm and breathing difficulties [12, 13]. Baclofen is a selective agonist of GABA B receptors, but at high doses it can also stimulate GABA A receptors. This effect was observed in both experimental groups. Emphysema was observed in the lungs of the experimental animals in both groups.

CONCLUSION

Thus, we observed a complex of pathological changes in the lungs (disturbances of blood circulation — venous, capillary plethora, hemorrhages into intraaveolar septa; emphysema, dystelectasis, WBC infiltration of intraalveolar septa). The changes were more severe 24 hours after the drug administration. This results along with the chemical analysis will be useful in establishing the fact of baclofen intoxication and the exact moment of the intoxication.

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HEMORHEOLOGIC PROFILE AND MICROCIRCULATORY HEMOSTASIS IN PATIENTS WITH CEREBROVASCULAR DISEASE IN DIABETES MELLITUS

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ABSTRACT — Diabetes mellitus is one of the most serious issues faced nowadays both by medicine and society in general, which is due to the wide spread of endocrine issues affecting nearly every country globally, the growing incidence rate, as well as the severity of complications that are hard to treat. Type 2 diabetes mellitus increases significantly the risk of developing acute cerebral blood circulation disorders, which urges further comprehensive studies focusing on the role played by the vascular-platelet relation and coagulation hemostasis in the development and progression of diabetic vascular complications. The results of our study, which involved 74 patients with acute cerebral circulation disorders against type 2 diabetes mellitus revealed alterations affecting the hemostasis system. This could be seen from activated vascular-platelet and coagulation links, decreased anticoagulant activity, and a slowdown in fibrinolysis. The severity of disorders induced by the alterations in the hemorheological profile and the microcirculatory hemostasis are associated with the duration of type 2 diabetes mellitus and the carbohydrate metabolism indicators (hyperglycemia, increased HbA1c levels and glycation end products).

KEYWORDS — diabetes mellitus; acute disturbance of cerebral circulation; blood rheological properties; hyperglycemia.

INTRODUCTION

The medical and social significance of cerebrovascular accident (CVA) is due to the respective significant rate of mortality, disability, persistent working incapacity, as well as socio-psychological maladjustment and economic losses manifested through treatment and rehabilitation costs [7, 14]. According to the Russian Statistics Agency (Rosstat) data, CVA accounts for 15.9% (2.9 cases per 1,000 inhabitants) in the overall number of deaths in the Russian Federation, giving way only to the deaths caused by cardiovascular issues. During that, the mortality rate resulting from brain vascular diseases is 4 times that observed in the USA and Canada [6]. One of the key risk factors for CVA development is type 2 diabetes mellitus (DM), whereas protein glycosylation, an increase in the plasma atherogenicity index, activation of coagulation and depression of blood fibrinolytic and anticoagulant properties, a reduction in the athrombogenic reserve, dysregulation of hemostasis and hemorheology occur under hyperglycemia. The basic mechanisms triggering cerebrovascular pathology in patients with type 2 diabetes are not only microcirculatory disorders, yet also small and large cerebral arteries atherosclerosis. It is notable that cell apoptosis, chronic vascular inflammation, activated intercellular adhesion molecules synthesis serve conditions for further progression of atherosclerotic lesions [1, 5, 9, 10].

Speaking of the pathogenesis related to later diabetic complications progress associated with microcirculation disorders and damage to the vascular bed of organs and systems, the authors point not only at structural and functional changes in the vascular wall, but at hemocoagulation and rheological disorders, too, the most sensitive being the erythrocyte and platelet link. Circulating platelets and erythrocytes are represented as populations featuring many different parameters (cell stability, cell age), while the transition from the physiologically normal status to the pathological one comes along with a change in the structure, properties of cell populations and their relationships [4, 13]. As respective literature holds it, patients with a long history of endocrinopathy, have hemorheological disorders manifesting through an increase in the plasma and whole blood viscosity, an increase in the red blood cells aggregation activity, erythrocyte membrane inability to resist destruction, low capacity of red blood cells for deformation, as well as exces-

sive development of leukocyte-erythrocyte aggregates [2, 3, 8, 11, 17].

Experts have shown that an increase in the glycated hemoglobin (HbA1c) levels, taken as a parameter for controlling carbohydrate metabolism and a marker of cardiovascular disorders, is an independent risk factor for CVA development, with females revealing a higher potential for its development compared to males [12, 16].

A significant number of works, both by national and foreign researchers, focus on early diagnosing of diabetic angiopathies, the development of algorithms for individual preclinical forecast, the choice of proper treatment tactics and prevention of vascular complications occurring through the toxic effect of hyperglycemia [15]. However, the data on the specific features pertaining to the population structure of peripheral blood platelets and erythrocytes, as well as data on the correlations of hematological parameters and the endocrinopathy duration in patients with type 2 diabetes and CVA, are of research interest, and are far from being complete and arranged in a systematic way.

Aim of study:
to analyze the status of the vascular-platelet link and peripheral blood coagulation hemostasis in patients with CVA in 2 diabetes mellitus.

MATERIALS AND METHODS

The study involved 74 patients (39 women, 35 men; mean age 63.7±11.2) with CVA and type 2 diabetes. The patients were admitted to the Neurology Ward of the Volgograd Regional Clinical Hospital #1 on the first day following the development of acute neurological symptoms. All patients underwent overall physical and neurological examination as well as general clinical and biochemical tests. The diagnosis of CVA was verified subject to the results of magnetic resonance imaging (MRI) and the clinical presentation. While the patient was undergoing the MRI procedure, the nature of the stroke (ischemic or hemorrhagic), the magnitude and prevalence of focal brain changes were clarified. The atherosclerosis degree affecting the head main arteries was identified and evaluated based on Duplex scanning. All the patients were divided into three groups: Group 1 — the history of type 2 diabetes ≤ 1 month; Group 2 — the history of type 2 diabetes — about 1 year; Group 3 — the history of type 2 diabetes ≥ 2 years. The diagnosis of DM was set subject to the WHO criteria (1999–2006) and the Russian specialized medical care algorithms for patients with diabetes mellitus (2017). On the day of admission, the patients were evaluated for reactive changes (blood smear test, stained by Romanovsky-Giemsa). The morphometric parameters of peripheral blood cells were studied using a Mekos-C3 hardware and software unit (light microscopy, ×1000). When identifying the HbA1c percentage, a Quo-Lab (EKF-diagnostic) glycosylated hemoglobin analyzer was employed. The hemostasis basic parameters were identified on an automatic Sysmex CS-2100i coagulometer.

The obtained data were processed employing the one-factor analysis of variance (ANOVA) with the Microsoft Excel 2019 software packages, while identifying the differences at the achieved level $p \leq 0.05$, provided that the observed Fisher criterion ($F_{obs.}$) exceeded the Fisher critical criterion ($F_{crit.}$). The analysis of the relationship between the poikilocytosis indicators and those of glycated hemoglobin was preformed using the Pearson (rP) and Spearman (rS) correlation coefficients.

RESULTS AND DISCUSSION

Of the total number of the examined patients, 91.9% were diagnosed with ischemic stroke; 8.1% — with hemorrhagic stroke; atherosclerotic lesions of the head main arteries were detected in 89.2% of the cases, while another 35.1% of patients were found to have hemodynamically significant stenoses (≥60%). Tables 1–3 offers a view at the indicators of the peripheral blood erythrocyte and platelet levels.

A comparative analysis of hematological indices of the erythron system (Table 1) points not only at a significant increase in the RDW (Red blood Cell Distribution Index) and HCT (Hematocrit number), yet also is indicative of an increasing anisocytosis and poikilocytosis, as well as a change in the plasma vs. red blood cell mass ratio towards an increase in the latter with an increase in the endocrinopathy history. The identified trend indicates accelerated cell aging under hypoxia and an increase in the peripheral blood viscosity. The other hematological parameters failed to reveal any significant change in the erythrocyte indices.

An analysis of peripheral blood preparations using the MEKOS-C3 units reveals a statistically significant ($P \leq 0.001$, $F_{obs.} > F_{crit.}$) increase in the anisocytosis coefficient (normocytes — 54%; microcytes — 44%; macrocytes — 2%) and poikilocytosis (echinocytes — 46%; acanthocytes — 28%; eliptocytes — 3%; discocytes — 15%; reversibly altered forms — 8%) (Fig. 1A). The data obtained from the erythrocyte formula have been confirmed through a significant increase in the diameter, area of red blood cells as well as a significant increase in their shape factor, which indicates progressive degenerative changes in the erythrocyte membrane due to lengthy metabolic disorders (increased HbA1c concentration, exposure to the lipid peroxidation system). Besides, an increase

Table 1. *Data from the hematological study of the peripheral blood erythrocyte link*

Indicators	group I n = 22	group II, n = 25	group III, n = 27	P at $F_{crit.} > F_{cr/criterion.}$
Hematology analyzer				
RBC ($\times 10^{12}$/l)	4,73±0,12	4,57±0,20	4,73±0,16	P≥0,5
MCV (fl)	89,8±0,7	89,2±1,4	91,9±1,9	P≥0,5
RDW (%)	13,5±0,3	14,9±0,4	15,9±0,3	P≤0,001
HCT (%)	38,2±0,9	40,3±0,9	42,6±1,0	P≥0,001
HGB (g/l)	145±7	145±4	151±5	P≥0,5
MCH (pg)	31±0,7	32±0,7	31,9±0,7	P≥0,5
MCHC (g/l)	350±7	344±2	344±1	P≥0,5
MEKOS-C3 hardware and software unit				
Ovalocytosis coefficient	0,84±0,02	0,83±0,01	0,84±0,01	P≥0,5
Poikilocytosis coefficient, (%)	9±1	16±1	44±3	P≤0,001
Anisocytosis coefficient (%)	7±0,4	7,5±0,4	10±0,4	P≤0,001
The area of red blood cells,(mm^2)	143,6±0,3	146,4±0,2	150,5±0,2	P≥0,05
The average diameter of the red blood cell (microns)	7,5±0,2	7,3±0,2	6,5±0,1	P≤0,05
Red blood cell form factor	33,6±1,2	40,9±1,0	43,4±1,0	P≤0,001

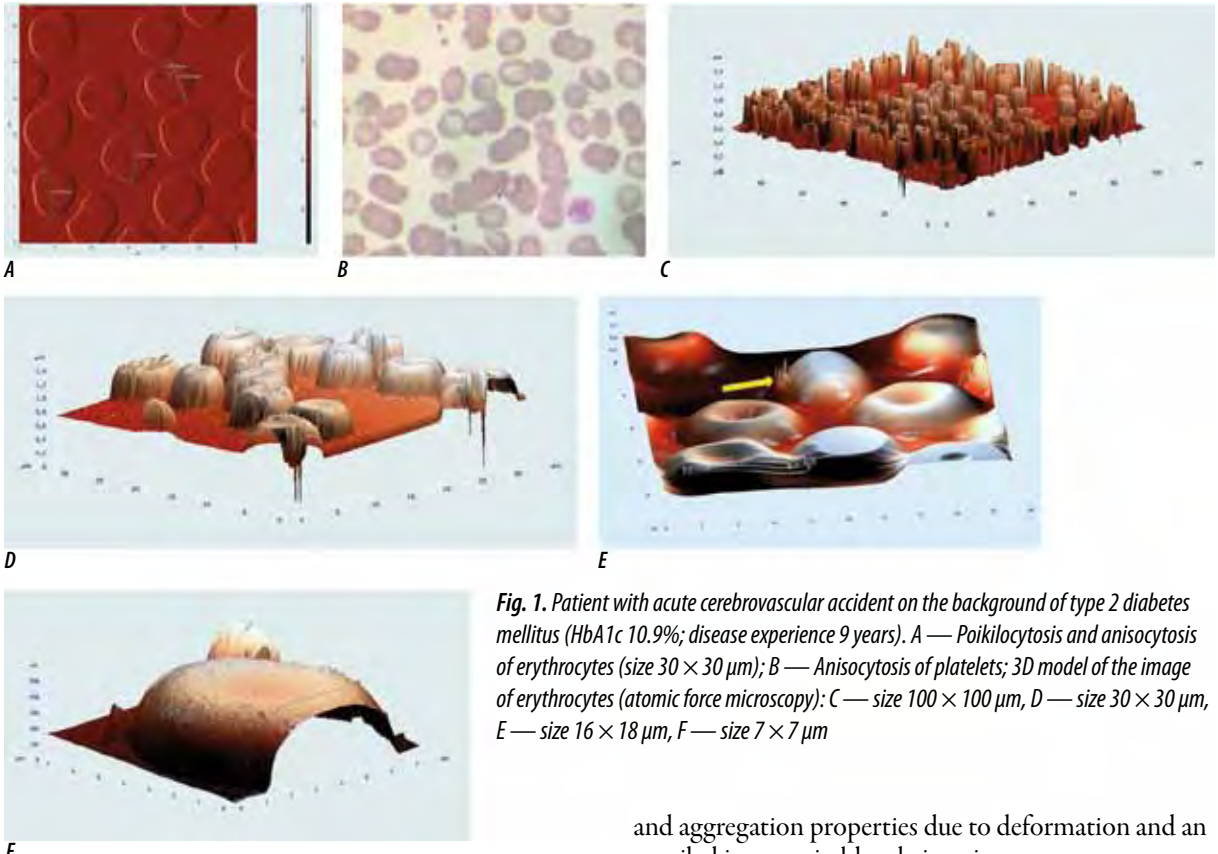

Fig. 1. *Patient with acute cerebrovascular accident on the background of type 2 diabetes mellitus (HbA1c 10.9%; disease experience 9 years). A — Poikilocytosis and anisocytosis of erythrocytes (size 30 × 30 μm); B — Anisocytosis of platelets; 3D model of the image of erythrocytes (atomic force microscopy): C — size 100 × 100 μm, D — size 30 × 30 μm, E — size 16 × 18 μm, F — size 7 × 7 μm*

in the number of aggregated forms of red blood cells and erythrocyte-platelet conglomerates was revealed (Fig. 1B), which means an increase in their adhesive and aggregation properties due to deformation and an entailed increase in blood viscosity.

A comparative assessment of platelet indices reveals a statistically significant increase in PCT (thrombocrit), PDW, MPV (average platelet volume), which points at an increasing thrombocytosis, a growth

in the degree of platelet anisocytosis, an increased number of their young and giant forms. This means an enhanced activation of the hemostasis system against an increase in the endocrinopathy history complicated by CVA.

A test of peripheral blood smears also showed high platelet anisocytosis (macrothrombocytes — 25%, normal platelets — 75%) (Fig. 2A, 2B) with their giant forms appearing in the decompensation stage in patients with different experience of endocrinopathy against CVA. The appearance of a large number of macrothrombocytes in patients with angiopathies and type 2 diabetes as early as starting from the 1st month of the disease progress means a high stress on the megakaryocytic germ of the red bone marrow, which results in immature hypo-granulated platelets separating from the megakaryocyte. The body's response involves compensating for the hypogranulation of platelets by their size, thus leading to the development of macrothrombocytes.

their aggregation delay stage decreases. An increase in platelet aggregates during ristocetin induction may be associated with an increased release of Willibrand factor from platelet α-granules, this leading to increased platelet agglutination.

In order to identify the correlations between an increase in the level of HbA1c and poikilo-, anisocytosis of red blood cells, an analysis of the respective relationship was carried out based on the Pearson coefficient (rP) and the Spearman coefficient (rS) (Table 3).

Following the Cheddock scale, a *strong* correlation was observed between the red blood cell poikilocytosis coefficient and the HbA1c level in Group III of patients, whereas in Group II the observed correlation could be described as *notable* (Table 3). High (strong) correlation was also to be observed in patients belonging to Groups II and III between the anisocytosis coefficient and the HbA1c level. The obtained outcomes indicate that patients with type 2 DM history

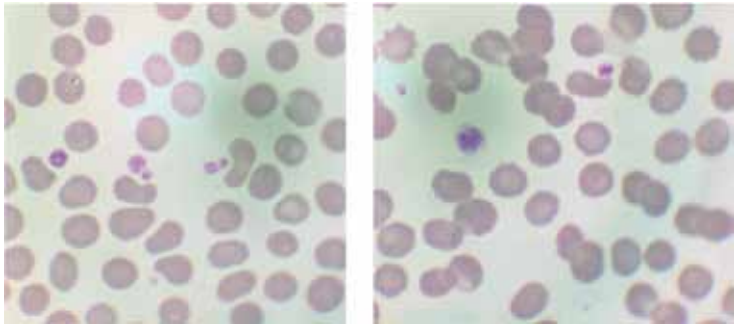

Fig.2. *Platelet anisocytosis in a patient with CVA and type 2 diabetes (HbA1c 7.9%)*

A statistically significant decrease in activated partial thromboplastin time (APTT) and an increase in the fibrinogen level in hyperfibrinogenemia in the groups being compared indicates hyperproduction of fibrinogen, as well as its accelerated conversion into fibrin, thus confirming increased activation of platelet α-granules, this leading to disturbance in the coagulation hemostasis parameters (Table 2). An analysis of induced platelet aggregation with various inducers revealed no significant change in the platelet aggregation degree, yet there was a significant increase in the platelet aggregates size to be observed with each of the inducers (ADP, collagen and ristocetin) (Table 2). An increase in the size of aggregates with ADP of 1 mmol/l and with collagen points at an increased readiness of platelet activation systems, due to which — when platelet aggregation with ADP is stimulated — absorption between aggregating elements grows; when collagen is stimulated, though, it results in an increase of the thrombocytes reactivity, whereas exceeding 1 year in the decompensation stage feature an increase in the aging process of the red blood cells functional pool with an increase in the HbA1c level, which leads to increased aggregation of red blood cells; increased blood viscosity and the development of micro-and macroangiopathies. It is to be noted that patients with CVA and a type 2 diabetes history exceeding one year, at an HbA1c level of up to 8%, have more significant anisocytosis of red blood cells, while patients with an HbA1c content of above 10% have poikilocytosis prevailing over anisocytosis. We believe that this condition is triggered by the launch of compensatory mechanisms aimed at eliminating hypoxia and maintaining homeostasis.

CONCLUSIONS

1. In patients with type 2 diabetes (where the history of the issue in questions exceeds one year) and CVA, changes in the peripheral blood rheological properties (red blood cells increased aggregation;

Table 2. *The hemostasis system and platelet aggregation indicators*

Indicators	group I n = 22	group II, n = 25	group III, n = 27	P at $F_{crit.} > F_{cr/criterion.}$
Indicators of the hemostasis system				
Activated Partial Thromboplastin Time (APTT), (sec)	36,3±1,2	35,8±0,5	34,2±0,3	P≥0,001
International Normalized Ratio (INR)	1,28±0,14	1,17±0,09	1,15±0,08	P≤0,5
Prothrombin time ratio, P/C ratio (%)	97±1	98±1	99±1	P≤0,05
Pro Time, PT (sec)	12,9±0,5	12,9±0,3	13,0±0,3	P≥0,5
Fibrinogen (gramm / liter)	4,20±0,2	4,52±0,18	5,77±0,16	P≤0,001
Platelet aggregation				
ADF 1mmol/l (%)	78±2	82±1	80±5	P≤0,5
Micron	8±0,01	8,4±0,4	11,9±0,9	P≤0,001
Collagen (%)	87±2	78±4	83±4	P≤0,5
Micron	8,1±0,4	8,9±0,4	11,1±0,6	P≤0,001
Ristomycin (ristocetin) (%)	89±3	93±2	98±3	P≤0,1
Micron	7,6±0,2	8,4±0,2	9,5±0,5	P≥0,001

Table 3. *The correlation coefficients of poikilo-, anisocytosis and glycated hemoglobin indicators*

Pearson coefficient		
	Dependence of HbA1c concentration on:	
	poikilocytosis	anisocytosis
Type 2 DM ≤ 1 month	<0,1	<0,1
Type 2 diabetes about 1 year	>0,6	>0,7
Type 2 DM ≥2 years	>0,9	>0,7
Spearman's Coefficient		
	Dependence of HbA1c concentration on:	
	poikilocytosis	anisocytosis
Type 2 DM ≤ 1 month	<0,1	<0,1
Type 2 DM about 1 year	>0,5	>0,7
Type 2 DM ≥2 years	>0,9	>0,7

red blood cells increased transformation into irreversible and reversible forms; plasma and whole blood increased viscosity; leukocyte-erythrocyte aggregates excess; increased adhesion of red blood cells to vascular walls endothelial cells) the accumulation of carbohydrate metabolism products shapes the basis of pathogenetic mechanisms that promote the development of later diabetic angiopathies.

2. In patients with type 2 diabetes, CVA mainly develops against significant atherosclerotic lesions of the head main arteries, including with hemodynamically significant stenoses.
3. CVA manifests a significant parallelism between the hemostatic system activation (changes in platelet aggregation, fibrinogen level, fibrinolytic activity) and the carbohydrate metabolism indices (plasma glucose, HbA1c, glycation end products).
4. An objective investigation of the HbA1c level, seen as a key diagnostic criterion for the CVA course in patients with carbohydrate metabolism disorders, will allow setting a timely diagnosis of endocrinopathy, as well as offering a forecast for the outcome.
5. A proper individualized pathogenetic treatment administered through the acute period of stroke will help improve the potential outcome and the life forecast for patients suffering from the said combined pathology.

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EMOTIONAL DISTRESS AS A RISK FACTOR FOR CORONARY HEART DISEASE IN CLINICALLY HEALTHY MEN WITH ALEXITHYMIA

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ABSTRACT — Objective: To identify emotional distress as a risk factor for development of coronary heart disease (CHD) in clinically healthy males with alexithymia. Materials and methods: We examined 89 clinically healthy males (mean age — 43.7 [39.0; 43.5] years) using Ch. Spielberger–Yu.Khanina tests, S. Subbotin and the Toronto Alexithymic Scale (TAS). Results: It was found that more than half of the respondents can be associated with alexithymia or they had its obvious clinical manifestations. As the severity of alexithymia increased, there was an increase in the levels of personal anxiety and a decrease in stress resistance that may increase the risk of developing coronary heart disease. Conclusion. Therefore, clinically healthy males with alexithymia of varying severity need psychological support to increase the effectiveness of primary prevention of CHD.

KEYWORDS — alexithymia, clinically healthy men, coronary heart disease, prevention, risk factors, anxiety, stress resistance.

INTRODUCTION

Along with other risk factors for the development of CHD in clinically healthy individuals, which is the leading cause of death in the adult population worldwide, emotional distress factors such as increased neuroticism, anxiety and alexithymia are of particular importance [1, 4, 5, 7, 9].

Goal

To identify emotional distress risk factors for CHD of clinically healthy males taking into account their tendency to develop alexithymia.

MATERIALS AND METHODS

The study included 89 clinically healthy male individuals (their average age is 43.7 [39.0; 43.5] years), since male sex is an independent risk factor for the development and complicated course of coronary heart disease [3.6]; all of them were blood donors. To identify the psychological characteristics of the individual, we used: The Toronto Alexithymic Scale (TAS) [2], questionnaires of Ch.Spielberger–Yu.Khanina for the assessment of reactive and personal anxiety [11] and S. Subbotina for the establishment of stress resistance levels [10]. The statistical analysis of the obtained data was carried out using the Statgraphics Centurion 19.1.2 program. The reliability of the statistical estimates used was at least 95%.

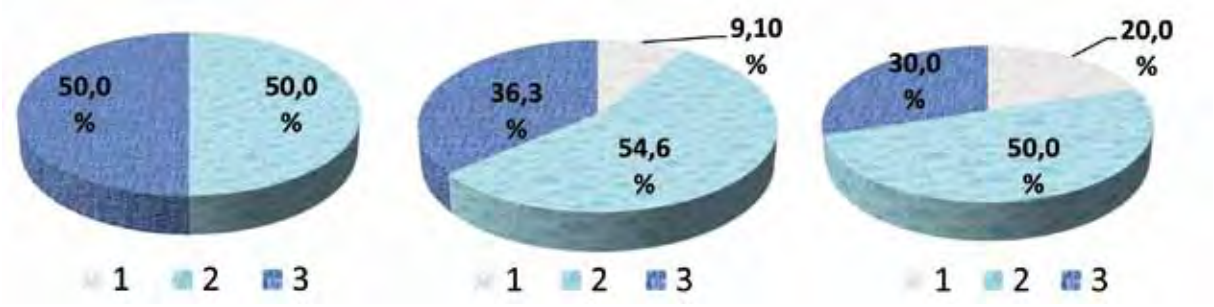
RESULTS

As a result of testing according to the data of the persons we observed, it was found that more than half of the respondents had signs of alexithymia of varying severity (20.9% were carriers of it, and 32.4% were in the *risk zone* of its development); the average indicators of this test in the general group were 64.1 points which corresponds to the *risk zone*.

Further psychological analysis of the healthy male individuals we observed was carried out taking into account the gradation according to TAS indicators: Subgroup I consisted of men with obvious clinical signs of alexithymia; subgroup II consisted of persons classified as *at risk* and subgroup III consisted of persons having indicators corresponding to the physiological norm.

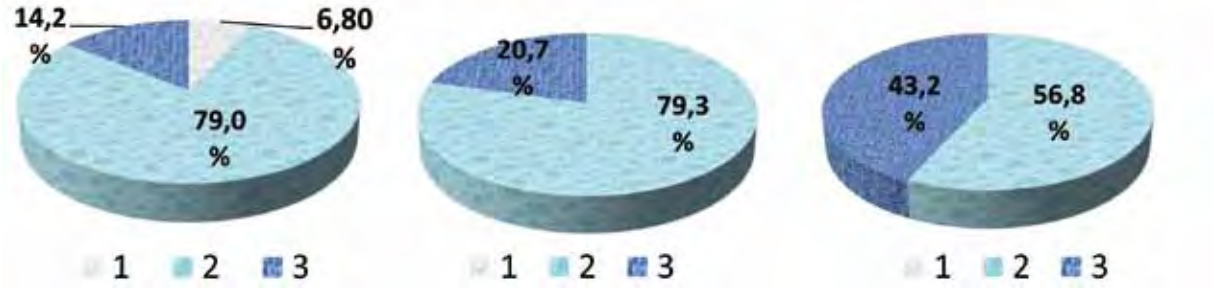
It was found that the carriers of alexithymia had indicators of personal anxiety at moderate and high levels (there was no low level of anxiety as a disposition); and respondents without deviations from the norm of TAS indicators, along with medium and high levels of anxiety as a disposition, had low personal anxiety in 1/5 of cases (Fig. 1, $p<0,05$).

Men who are not resistant to the action of stress agents were found only in the subgroup of carriers of alexithymia, and the largest proportion of respondents with high stress resistance was detected in the subgroup of clinically healthy men without signs of alexithymia (Fig. 2, $p<0,05$).



A B C

Fig. 1. *Distribution of indicators of personal anxiety in the individuals observed in the context of alexithymia*
Note. *Values of anxiety as a disposition: A — for carriers of alexithymia; B — for persons who are at "risk" of developing alexithymia; C — in the absence of deviations from the norm of TAS indicators. Levels of personal anxiety: 1 — low; 2 — moderate; 3 — high*



A B C

Fig. 2. *Distribution of indicators of resistance to the action of stress agents in the individuals observed in the context alexithymia.*
Note. *Values of resistance to the action of stress agents: A — for carriers of alexithymia; B — for persons who are at "risk" of developing alexithymia; C — in the absence of deviations from the norm of TAS indicators. Levels of resistance to the action of stress agents: 1 — low (unstable); 2 — medium; 3 — high*

DISCUSSION OF THE RESULTS

Alexithymia, as a psycho-emotional characteristic of a person and an independent risk factor for the development of neuroses and psychosomatic pathology, including cardiovascular (and, first of all, CHD), is increasingly found not only in various psychosomatic diseases, but also in clinically healthy individuals [8, 9]. So, in our study, in clinically healthy men who are blood donors, the average test results for TAS allowed them to be attributed to the *risk group* for the development of alexithymia. During detailing the results of this test, the predominance of male individuals with a tendency to develop alexithymia and its clinically obvious manifestations over healthy men whose test indicators did not exceed the physiological norm was established. Based on this, it was interesting to look at other independent emotionally negative risk factors for CHD of people under our supervision (such as reduced stress tolerance and increased anxiety) through the prism of alexithymia, for which all respondents were divided into three subgroups, depending on the presence and severity of alexithymia in them. This approach proved to be justified since it allowed us to identify the following trends: as the severity of alexithymia increased, there was an increase in the level of anxiety as a disposition and a decrease in the level of resistance to the action of stress agents in the individuals we examined. The abovementioned increases the relevance of the expediency of the psychological support for clinically healthy men who either have a tendency to develop alexithymia or its obvious clinical signs.

CONCLUSION

Thus, as the severity of alexithymia increased, there was an increase in the level of anxiety as a disposition and a decrease in the level of resistance to the action of stress agents in the healthy male respondents

we observed which may increase the risk of developing CHD in them. Therefore, clinically healthy individuals who have a tendency to develop alexithymia or its obvious manifestations need psychological correction of the personal changes established in them to increase the effectiveness of primary prevention of CHD.

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THE DIAGNOSTIC ROLE OF FETAL HEMOGLOBIN AND BLOOD OXYGEN SATURATION IN CHRONIC LIVER DISEASES

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ABSTRACT — This research aims to study levels of fetal hemoglobin and blood oxygen saturation in 264 patients with chronic diffuse liver diseases, among them 69 patients with chronic hepatitis and 195 patients with liver cirrhosis. To determine the levels of fetal hemoglobin, a patented method of rocket electrophoresis in agar gel with sodium dodecyl sulfate was used. The level of fetal hemoglobin in liver cirrhosis were significantly increased compared to chronic hepatitis, and, in both cases, compared with the control group. Oxygen saturation of the blood was reduced mainly in liver cirrhosis. In patients with liver cirrhosis a relationship between fetal hemoglobin and oxygen saturation was established. Our study helps in the early diagnosis of latent chronic hypoxia and hypoxemia in patients with liver disease. This allows to assess disease severity and adjust the treatment.

KEYWORDS — chronic hepatitis, liver cirrhosis, fetal hemoglobin, oxygen saturation, hypoxemia.

INTRODUCTION

The role of hypoxia and hypoxemia in Chronic Liver Diseases (CLD) has been well investigated by researchers. [1, 2, 3]. Hypoxia and hypoxemia in Chronic Hepatitis (CH) and Liver Cirrhosis (LC) can be both local and systemic. Inadequate oxygen supply of liver parenchyma may occur due to high vascular resistance, intrahepatic shunts, intravascular thrombosis, reduction of the area of sinusoidal capillaries [2, 3]. In turn, hypoxia and hypoxemia, becoming chronic, contribute to the progression of fibrosis and LC, deterioration in the course and prognosis of the disease [2, 4, 5].

Fetal hemoglobin (HbF) is the dominant form of hemoglobin present in the fetus during gestation. HbF is produced by erythroid precursor cells from 10 to 12 weeks of pregnancy through the first six months of postnatal life. Yet, it is also considered as one of the markers of tissue hypoxia in adults. At the same partial pressure, HbF more actively absorbs oxygen and gives carbon dioxide than adult hemoglobin (Hb) [6, 7]. Normal HbF is found in adult blood minimum concentration of 1–1.5% [7, 8]. The increase of its content, detected in a number of diseases of internal organs, is considered as an adaptive reaction of the organism, aimed at stabilization of tissue gas exchange [7]. Previously, we described the HbF concentration increase in CLD [8].

Objective: to improve the diagnosis of hypoxia and hypoxemia in CH and LC based on the study of changes in blood levels of HbF and the degree of blood oxygen saturation.

MATERIALS AND METHODS

The study included 264 patients with CLD (138 men and 126 women aged 20 to 60 years). With CH was observed 69, with LC — 195 patients. Control group (CG) consisted of 50 healthy donors matched for age and gender.

Patients were examined in the hospital at the stage of exacerbation of their underlying disease. CH and LC was diagnosed on the basis of complaints of patients, anamnestic and clinical data, the results of laboratory and instrumental methods of examination. The diagnosis was made in accordance with the existing classifications of CH and LC.

We used individual quantitative analysis of HbF, a patented rocket electrophoresis technique in an agar gel with sodium dodecyl sulfate, in all patients with CH, LC and CG. The blood oxygen saturation (SpO2) was determined in 58 patients with CH, 115 — LC and 50 patients CG using the pulse oximeter "RM S-31". Statistical data processing was performed by the "Statistica 10.0" application package.

RESULTS AND DISCUSSION

The average values of fetal hemoglobin in patients with CH, LC and CG are presented in the Table 1.

The concentration of HbF in CG was 2.4 [1.4; 2.9] g/l, which generally corresponds to the literature data. There were no gender differences in the CG. The mean HbF concentrations measured in absolute value in the CH and LC groups did not differ significantly (3,4 [2; 4.5] g/l and 3.6 [2.4; 4.3] g/l, respectively;

Table 1. *Mean values of fetal hemoglobin in patients with chronic hepatitis, liver cirrhosis and the control group*

Indicator	Control group (n=50)	Chronic hepatitis (n=69)	Liver cirrhosis (n=195)
HbF (g/l)	2.4 [1.4; 2.9]	3,4 [2; 4.5]*	3.6 [2.4; 4.3] *
Total Hb (g/l)	143 [125; 165]	133.7[115;155]**	118.6 [90; 134]*
Percentage of HbF of total Hb (%)	1.53 [0.5; 1.7]	2.6 [1.8; 3.7]* **	3.4 [2.7; 5] *

** — the reliability of differences between patients with CH and LC compare CG<0,01*
*** — the reliability of differences between patients with CH and LC <0.01*

p>0.05), but were significantly higher than in CG (p<0.01).

Due to the frequent development of anemic syndrome in CLD, lower average values of total Hb are observed in CLD patient, compared to CG. Therefore, for an objective analysis of the data and their reliable verification, the absolute values of HbF (g/l) were converted to the percentage of HbF (%) of total Hb.

It was shown that after the transfer of the mean values of HbF in absolute values to their percentage to total Hb, there were significant differences (p<0.01) between the groups of patients with CH and LC, there was also an increase in the reliability of differences between CH and LC patients to CG.

The increase in fetal hemoglobin levels in CH and LC can be explained by the fact that, as a chromo protein evolutionarily adapted to the stabilization of tissue gas exchange in chronic hypoxia with more affinity for oxygen than adult hemoglobin, HbF reacts to chronic tissue hypoxia in liver disease. The increase in the concentration of HbF in erythrocytes occurs due to the development of adaptive reactions of erythron in hypoxia and is associated with partial depression γ-chain globin in the background of intense erythropoiesis.

To establish possible relationships of the concentrations of HbF with indicators of blood oxygen saturation in 58 patients with CH, 115 with LC, and 50 patients of CG were determined SpO_2 values.

The average level of SpO_2 in CG amounted to 98.6±0.5%. Variability values were in the range 97–99%.

The SpO_2 level in the group of patients with CH averaged 96.6±0.7%, which did not differ significantly from the norm. The mean value of SpO_2 in the group of patients with LC was 94.9±0.7%, which was significantly lower (p<0.05), compared with CH (98.6±0.5%). It should be noted that the revealed hypoxemia in the majority of patients with LC was weakly expressed: the variability of the level of SpO_2 in the LC was in the range of 92–97%.

Analysis of the study results showed that at normal values of SpO_2≥95%, the content of HbF% averaged 2.98±0.13%, while at SpO_2<95% — 3.35±0.12% (p=0.04). Consequently, with a decrease in blood oxygen saturation characterizing the development of signs of hypoxemia in LC patients, the concentration of HbF% was significantly higher than in normal indicators of SpO_2.

CONCLUSION

Detection of increased values of tissue hypoxia marker HbF and reduced blood oxygen saturation indicate the development of hypoxia and hypoxemia in CH and LC. Therefore, the study of the indicators of HbF and SpO_2 increases the effectiveness of early diagnosis of latent chronic hypoxia and hypoxemia in clinical practice for the chronic liver disease. These methods enable improving the diagnosis of disease severity and adjusting the treatment.

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ANALYSIS OF TISSUE EOSINOPHILS IN THE STRUCTURE OF GASTRIC POLYPS

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ABSTRACT — A study of the diagnostic value of tissue eosinophil infiltration in the structure of polyps of the human gastrointestinal tract and surrounding tissues was carried out. We investigated intestinal biopsies from 189 patients aged 40–90 years with polyps, cancer, metastases. This retrospective study allows us to note the possibility of using eosinophils in the development of criteria for early malignancy and a promising outcome of neoplasms in the mucous membrane. Eosinophils are important players in intercellular interactions within the polyp structure and the tissues around it. The complete absence of eosinophils in the tissue of malignant polyps indicates the malignancy of the neoplasm. We revealed relationship between apoptosis and eosinophilic infiltration; both of them affect the outcome and prognosis of polyp development. Apoptosis induction by eosinophils and programmed cambium necrosis in tumor progression need to be further researched.

KEYWORDS — human gastrointestinal tract, polyps, malignancy, mucous membrane, monocytes / macrophages, eosinophils, colon; gastrointestinal disease; neoplasms.

INTRODUCTION

The aggregate indicator of the prevalence of oncopathology in Russia reaches 2.399% with an annual increase of 1.7%. In the Primorsky Krai (Russian Far East. 1.9 million population), in 2016 only, gastric cancer was diagnosed in 493 people [1]. However, the level of early diagnosis of malignant polyps in stomach remains very low. Detected malignant neoplasms are confirmed morphologically in 91.3% of cases, which indicates a late diagnosis of malignancy [2]. One of the tasks of solving the problem of early diagnosis and prevention of cancer is the development of morphological criteria for the malignancy of benign formations in the structure of the gastrointestinal tract and the strategy of pathogenetically substantiated treatment of neoplasms for the prevention of their malignancy and relapses.

According to Chetty R. (2016), at the present stage, the most discussed are the mechanisms of dysplasia, as well as issues related to the nomenclature, diagnostic criteria, histological variants, coexistence with other types of polyps, and those differential diagnosis [3]. The exceptional immunological properties of eosinophils and their ability to induce tissue remodeling, which is especially important in the destruction and disruption of cellular interactions in the system of epithelial-mesenchymal tissues during carcinogenesis, puts the study of eosinophilic infiltration during tissue malignancy at the forefront of relevance [4, 5]. Cansiz Ersöz C., Kiremitci S., Savas B., Ensari A .. (2020) recognize eosinophilia against the background of polyps in 88% with high sensitivity and specificity in predicting carcinogenesis [6]. Clinical and morphological studies in the dynamics of treatment for patients with polyps in the gastrointestinal tract are necessary to elucidate the morphological criteria of malignancy and provide pathogenetically based recommendations for assessing the risk and predicting the outcome of benign polyps.

Aim of study:

To investigate eosinophils localization among the cellular phenotypes of myeloid cells in the structure of polyps in various parts of the human gastrointestinal tract.

MATERIAL AND METHODS

Analysis of the clinical material based on the study of 189 patients aged 40 to 90 years treated at surgical departments of hospitals in the Primorsky Krai (Russia) 2018 to 2020 were carried out. Clinical material was obtained with informed consent of the patients and in accordance to the Helsinki Declaration (Revision 2013). The study was approved by Ethic Committee of Far Eastern Federal University (Vladivostok, Russia).

Immunohistochemical methods for processing biopsies of polyps from various parts of the gastrointestinal tract using monoclonal antibodies (MCA) (clone KP1, code No. M 0814, lot 119) were used. Macrophages were also identified by the CD68 marker

(a highly glycosylated transmembrane glycoprotein that is localized in lysosomes). Molecular clone CD68 showed that LGP (a family of lysosomal glycoproteins) with plasma membrane proteins play a role in lysosomal traffic and endocytosis, including lysosomal membrane protein-1 and 2 associations (LAMP-1 and LAMP-2). For labeling CD163 used clone 10 D6, class of immunoglobulins IgG1. The antibodies were labeled with horseradish peroxidase and diaminobenzidine was used to visualize the binding. Cleansing and detection of antigenic determinants was carried out in a glass container filled with a reducing solution and creating a water bath for 1 hour. Some of the preparations were processed using microwave radiation, which gives the best unmasking effect, within half an hour. Used to unmask antigens 10 mmol/L citrate buffer, pH 6.0 or DAKO TRS (Target retrieval solution, code No. S 1700) The cooled preparations were washed in distilled water. Antibodies were used at a dilution of 1:50 and 1: 100. Brown staining indicated a positive reaction.

RESULTS AND DISCUSSION.

Analysis of clinical material and case histories in patients showed that against the background of cancer in various localization of the gastrointestinal tract, the stomach is in the lead, cancer of which in 2016 amounted to 199 cases per 100,000 population, which was 2 times higher than the intermediate data for Russia (19.83 versus 9.73). The share of the colon accounts for 451 cases, which amounted to 25.71 cases per 100 thousand of the population; rectal cancer was 294 cases (146 per 100 thousand population) [1]. In men, the number of patients with polyps in the group of 31–40 years old is 57%, 41–50 years old — 40%, 51–65 years old — 61%, 66–70 years old — 22%, 72–74 years old — 71%. In the examined women with clinical manifestations at the age of 40–50, polyps were found in 100%; in the 51–65 age group — polyps are identified in 40%; at 66–70 years old — at 25%; there are no women with polyps in the group over 70 years old among the examined patients (Fig. 1)

Polyps were more often detected by endoscopy, mainly in the large intestine, in both men and women. Cases of malignancy are established both by clinical diagnosis and histologically confirmed.

Surgical treatment was performed in 95% of patients, conservative treatment — in 2% of patients, the outcome was favorable, the prognosis was positive in 93% of patients. The use of the fast track surgery (FTS) protocol — *accelerated recovery after surgical operations* in combination with the laparoscopic approach made it easier for patients to tolerate surgical treatment and leave the hospital 5–6 days after the operation. Processed and analyzed biopsies of the gastrointestinal mucosa were obtained from 189 patients, 112 men and 77 women) aged 40 to 90 years, who had polyps, cancer, metastases. (Table 1).

A higher relative number of male patients among patients with pathology of gastrointestinal mucosa indicate a higher incidence of morbidity and mortality in the male population. The distribution of patients in Primorsky Krai with clinical manifestations and identified pathology of the gastrointestinal tract is shown in figure 2.

Age-related changes in the structure of the gastrointestinal mucosa are also characterized by the fact that almost all sections from biopsies obtained for the purpose of diagnostic measures revealed the presence of leukocytes infiltration around the vessels in the connective tissue of the lamina propria and submucosa. At the same time, in the composition of the infiltrate, eosinophils were identified in large quantities. In the area around the polyp, various types of eosinophils are present. In the first group, eosinophils have a clearly identifiable cytolemma, a nucleus of a round, oval or irregular shape. Another type is represented by degranulating cells with a nucleus of two or one fragments. Eosinophils were identified not only in the lamina propria, but also penetrated into the basal epithelial layers (Fig. 3).

Within the changes of epithelium of the mucous membrane in the structure of the polyp and its metaplasia, eosinophils were absent in our observations. The morphological findings were process eosinophils with signs of degranulation identified in the epithelial lamina with abnormally wide space involved in to apoptosis. As part of the infiltrate, eosinophils are grouped near the stem migrants, near the vessels and inside the preserved blood vessels. In the area surrounding degranulating eosinophils, cells with signs of apoptosis are observed (Fig. 4).

In addition, immunohistochemical study showed immunoreactivity to CD4, CD8, CD68, CD163, which were observed in all studied biopsies of polyps and their surrounding tissue (Fig. 5).

These results suggest that CD4, CD8, CD68, CD163 are key markers for clinical diagnosis and prediction of polyps malignancy in the structure of the gastrointestinal tract. As well as eosinophils has unlimited importance in the analysis and prognosis of polyps malignancy based on a thorough analysis of preparations stained with hematoxylin and eosin with immunostained sections.

We noted that with the formation of a polyp on the surface of the mucous membrane, the morphological picture of the altered tissue is characterized by deformation of the basement membrane, apoptosis

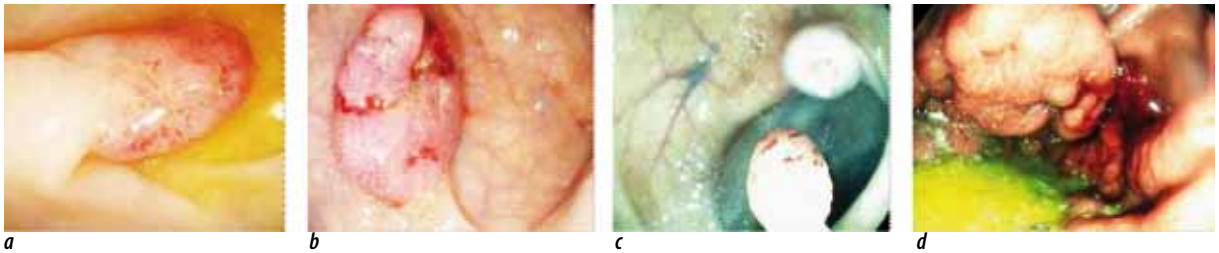

Fig. 1. Endoscopic examination of patients with gastric mucosa. a) 65-year-old man patient. Gastric polyp. b) 67-year-old man patient. Gastric polyp a; c) 72-year-old man patient. Gastric cancer. d) 75-year-old man patient. Gastric cancer.

Table 1. Distribution of material according to the revealed pathology

Age (years old)	Patients (number)	Disease		
		Polyps	Cancer	Metastasis
40–49	6	4	1	1
50–59	9	7	2	0
60–74	85	64	21	1
75	81	66	15	2
90	8	4	4	2
Total:	189	140	43	6
Total: %	100%	71%	26%	3%

Fig. 2. Distribution of older age groups of patients with complaints by the presence of pathology

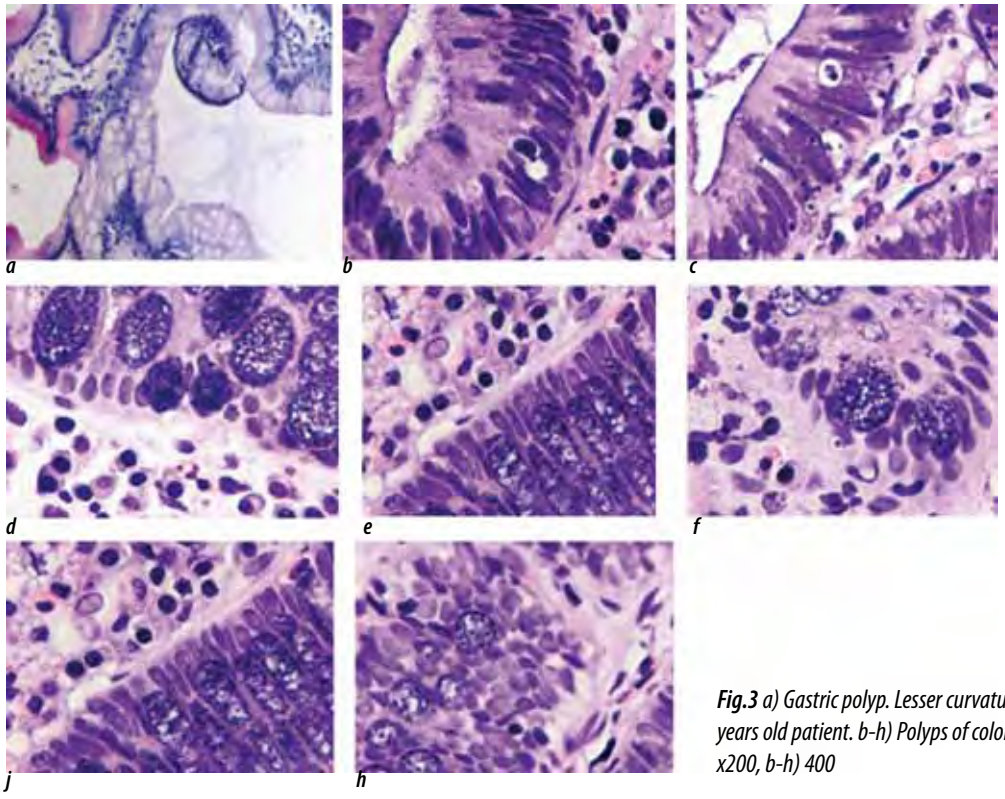

Fig.3 a) Gastric polyp. Lesser curvature of the stomach. 64 years old patient. b-h) Polyps of colon. H/e staining. Zoom a) x200, b-h) 400

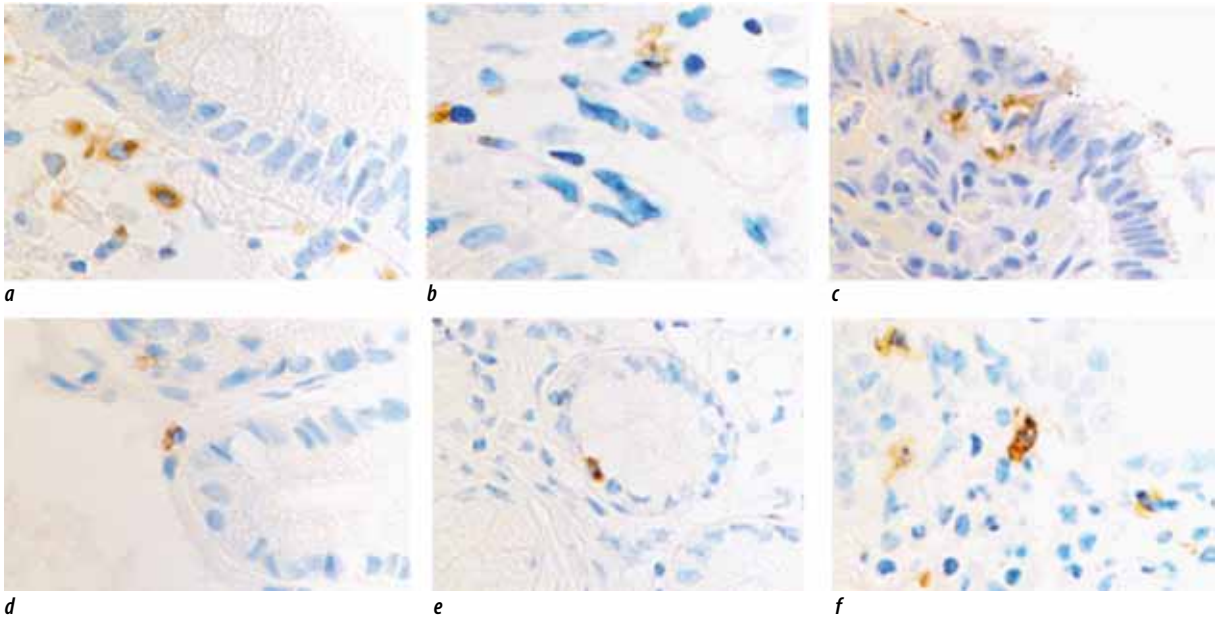

Fig. 4. *Colon polyps. Immune staining. a) CD4; b) CD68; c) CD68; d,e,f) CD163. Zoom ×400*

of epithelial cells and infiltration of migrant cells from the blood into the area of altered tissue. Identified cells of the infiltrate proliferate by amitosis, can be identified both near the epithelial plate, and at the site of destroyed epithelial cells, as well as outside the epithelium on the surface. The structure of the lamina propria of the mucous membrane is not identified, which may be associated with both the death of connective tissue cells responsible for the production of intercellular substance and their veiling with elements of a blood infiltrate containing migrant cells.

The long-term process of polyp formation is accompanied by apoptosis, folding of the basement membrane, and complete disruption of epithelial restitution. This indicates the peculiarities of reparative processes in the structures of the mucous membrane against the background of the formation of and their malignancy. We found that eosinophils in the surrounding polyps tissue are identified in 100%, and under conditions of malignancy, their number decreases by 20%. The number of cells in the monocyte pool also decreasing. But it is worth paying attention to the pathological forms of eosinophils, which do not normally occur in peripheral blood or tissues. A common eosinophil has a nucleus consisting of 2 segments; in our studies, the eosinophilic population is represented by cells with spherical, sometimes eccentric nuclei.

The second phenotype of eosinophils had nuclei of two segments, but also spherical in shape, while normally the segments of the nuclei of eosinophilic granulocytes have a small width of 12–20 μm and a length of up to 7 μm. In our observations, the nuclei were dumbbell-shaped, but the venom chromatin was unevenly distributed. This may reflect not only quantitative, but also qualitative changes in protein synthesis and function in general. Caruso R.A., Branca G., Fedele F. et al. (2014), consider eosinophilic infiltration in the stroma of a gastric tumor associated with carcinoma [8]. But these data about the participation of eosinophils in the destruction of normal tissue structures and the synthesis of stromal elements of the tumor require additional research.

We noted that the eosinophilic population spreads from the lamina propria to the cambial layer of the epithelium, with corresponding co-localization with CD4, CD8, CD68 and CD163 positive cells, which indirectly indicates that in the case of polyps formation, eosinophils are able to acquire the properties of antigen-presenting cells, as well as macrophages. Beltran M, Khurana S, Gil Y, Lewis JT, Kumar R, Foran JM. (2020) described that tubular adenomas with dense transmural inflammatory infiltrates, which mainly consist of eosinophils and store Charcot-Leiden crystals of histiocytes, in some cases are preceded by parasitic infection, which leads to a high content of eosinophils in the leukocyte infiltrate of the forming polyps and their environment [9].

Cai L, Chen Y, Xiao SY. (2020), on the contrary, concider that eosinophilia in the tissue does not always occur against the background of parasite invasion [10]. According to our analysis of the anamnestic data, there were no parasitic infections in the patients. We believe

that the migration activity of eosinophils to the focus of polyp formation may be associated exclusively with a violation of signal interactions in the system of local immune homeostasis of the gastrointestinal mucosa.

Agaimy A., Mudter J., Märkl B., Chetty R. 2011) in two cases of polyps of cytomegalovirus etiology, tissue eosinophils were observed in the mucous membrane of the gastrointestinal tract [11]. Therefore, it can be assumed that the threshold values of the number of tissue eosinophils in the gastrointestinal mucosa against the background of neoplasms require further research, since they may be important in the differential diagnosis of the etiology of polyps.

Moezzi J, Gopalswamy N, Haas RJ Jr, (2000) observed in 75% of all adenomas from mild to moderate eosinophilia, especially pronounced in the tissue surrounding the polyp and their absence in the stroma of invasive adenocarcinomas [12]. Similar data obtained in our study indicate that the quantitative dynamics of tissue eosinophils can be taken into account in the development of a treatment strategy and influence the prognosis of polyp outcome in clinical assessment.

It should be noted that over the 20th and 21st centuries, many microorganisms had laurels of a starting source in the pathogenesis of cancer, but the absence of 100% oncogenesis in all carriers of these microorganisms [13], as well as spontaneous recovery in 98% in the presence, for example, in polyps of papilloma virus, which is currently recognized as a trigger in the development of cancer with localization in the mucous membranes of the gastrointestinal tract, indicate that the key pathogenetically significant process in the oncogenesis of the gastrointestinal tract is a process unknown today [14, 15].

The almost complete lack of data on intercellular signaling interactions in the stem niches of the gastrointestinal mucosa of patients with confirmed polyp etiology is an obstacle to the introduction of cellular technologies in gastroenterology and oncology [16, 17]. Although a routine biopsy is recommended in accordance with the Sydney system, this is not enough for an adequate assessment of the pathological process, since the sampling zones are actually endoscopically distinguishable areas, excluding the surrounding tissues from the complete analysis. Therefore, histological studies in areas at the border of the polyp zones and unaltered macroscopically tissue are of high importance. The persistence of dyspeptic symptoms in patients after therapy or the absence of symptoms after extirpation of polyps in patients dictate the search for more evidence-based criteria for the risk of developing gastrointestinal cancer. In cancer, the presence and degree of penetration of eosinophilic granulocytes into malignant tissue can provide important prognostic information [18].

We have found that the formation of polyps is associated, first of all, with damage of epithelial restitution, increased proliferative activity, accompanied by depletion of the cambial stem elements of the integumentary epithelium. Migration of tissue eosinophils is required for adaptive tissue remodeling in the structure of the polyp and its environment [19]. We noted that with the formation of a polyp on the surface of the mucous membrane, the morphological picture of the altered tissue is characterized by the disappearance of the basement membrane, apoptosis of epithelial cells and migration of immunocompetent cells from the blood to the zone of altered tissue.

Ieni A, Branca G, Parisi A, (2015) in patients over 72 years of age, a distinctive morphological sign of malignancy is associated with an increase in neutrophil infiltration, although the true mechanism of this process is also unclear [20].

CONCLUSIONS

The mechanism of HPV-induced carcinogenesis, despite the widespread nature of this infection, has not yet been elucidated and substantiated. Based on the integral assessment and comparative analysis of pathological changes in the gastrointestinal mucosa, there are still no definite definitive morphological markers of epithelial metaplasia of polyps and malignancy, as well as no explanation for malignancy of only 1% of polyps, while tumors are often found near polyps. These facts indicate that local immune homeostasis plays a role in malignancy, which must be guided by when making a diagnosis, predicting the outcome of polyps, determining the scope of surgical intervention, and postoperative treatment strategies.

An increase in the number of eosinophils in the general inflammatory cellular spectrum in the area of malignancy is an autonomous, antitumor mechanism, the decoding of which can contribute to the development of conservative methods for the treatment of gastric polyps. The obtained data open up prospects for the development of a new strategy for the correction of malignancy using the secretory activity of eosinophils by inducing an increase in the number of required phenotypes. One of the focuses of targeted treatment and prevention of malignancy is the eosinophilic pool of leukocyte infiltrate.

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EVALUATING EXPERIENCE WITH ANORECTAL MANOMETRY IN AN OUTPATIENT SETTING

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ABSTRACT — Purpose of the study: 1) Evaluating application of anorectal manometry in an outpatient setting. 2) Determination of reference manometric and point criteria for normal rectal sphincter indicators and Fecal Incontinence (FI) according to anorectal manometry data using a Peritron anal sensor.
Materials and methods: The retrospective study was based on the results of anorectal manometry using Peritron equipment (Cardio Design Pty Ltd, Australia) on 1200 patients with various proctological diseases, who were examined and treated at the Center for Outpatient Proctology of the surgical department of Railway Clinical Hospital (Rostov-on-Don, Russia) from 2015 to 2020. All patients were divided into 4 groups in accordance with the clinical classification of Fecal Incontinence (FI) developed at the National Coloproctology Research Center and the obtained results. As a subjective assessment, the Cleveland Fecal Incontinence Scale (Wexner) was used, and objectively, the results of anorectal manometry using the Peritron sphinctometer.
Results: Our manometric data enabled to develop a reliable indicator scale of normal sphincter function and various degrees of fecal incontinence according to the Peritron findings and allowed to recommend the device for a wider introduction into clinical practice, in particular in coloproctology, to perform anorectal manometry.

KEYWORDS — anorectal manometry (ARM), sphinctometer, rectal obturator, Peritron.

RELEVANCE

One of the important issues a coloproctologist faces in his clinical practice during the initial, pre and postoperative patient examination is to determine the parameters of the muscular group condition of the pelvic floor, its obturator function, in particular, bowel incontinence with gas and fecal leaks.

Literature references state that the process of retention of gases and feces is multifactorial. It depends on the coordination of the following factors:

— *ideal stool* formation factors: volume and quantitative indicators of feces — diet, adherence to dietary recommendations of meal composition, intake of a sufficient amount of plant fibers, water balance, etc.

— anatomical factors: the geometric position of all parts of the colon and in particular the anorectal angle, etc.;

— motor-evacuation function factors: coordination of peristaltic and antiperistatic waves, sufficient evacuation force, etc.;

— neurological factors: the integrity of the receptor apparatus and the conducting nerve pathways of the rectum and the anal canal, the coordination of spinal cord structures and the brain with the muscular structures of the external and internal rectal sphincters, observance of the temporary fecal flow consistency, the absence of stress effects;

— the presence and severity of proctological diseases of the anal canal and rectum: anal fissures, paraproctitis, cryptitis, sphincteritis, chronic colitis, etc.

But many authors mostly attribute the retention of gases and feces to the proper functioning of the rectal sphincters due to the pressure created by the smooth muscles of the internal sphincter (70–80%), and striated muscles of the external sphincter (20–30%). [2, 3]

Today, there are many scales that allow a subjective assessment of the state of gas and fecal incontinence in proctological patients. Most authors, when interviewing a coloproctological patient for a subjective assessment of incontinence, use the Cleveland Fecal Incontinence Scale (Wexner) (Wexner Anal Incontinence Scale) (Table 1). [4]

But the subjective nature of the indicators, the point-based interpretation of the parameters, ranging from 0 = full control to 20 = complete incontinence, in conjecture with a lack of understanding of intermediate values and their relationship with the degree of incontinence, as well as possible *errors* due to personal parameters such as wearing pads and lifestyle changes, do not always allow to adequately assess the initial state and the conditions after surgical treatment and rehabilitation therapy.

An objective method for assessing the state of incontinence of gases and feces in proctological patients is considered to be instrumental measurement — anorectal manometry. At the moment, there is no list

Table 1. *Interpretation of parameters: 0 = full control, 20 = complete incontinence*

Cleveland Fecal Incontinence Scale (Wexner) (Wexner anal incontinence scale)					
	Frequency				
Incontinence type	Never	Rarely (< 1 once a month)	Sometimes (< 1 once a week, but > 1 once a month)	Usually (< 1 once a day, but > 1 once a week)	Always (> 1 once a day)
Hard stools	0	1	2	3	4
Loose stools	0	1	2	3	4
Gases	0	1	2	3	4
Wearing pads	0	1	2	3	4
Lifestyle change	0	1	2	3	4

Interpretation of parameters: 0 = full control, 20 = complete incontinence

of sphincterometry devices recommended for mandatory use in the Russian Federation. Different manufacturers offer their own models for ARM. Currently, 3 devices with sphincterometry functions are registered in the Russian Federation: 1 — sphincterometer SF-01 (Gastroscan-SF), and 2 — multifunctional manometric complex WPM Solar (MMS, Netherlands) and sphincterometer S 4402 MCM "Pro Medica GmbH", Germany [2, 3]. Unfortunately, in spite of the undoubted functionality of these models, an important limiting factor for their widespread use by both doctors and patients remains the cost. One possible way to avoid this situation is using Peritron equipment to perform anorectal manometry. It has all the necessary functionality at a reasonable price. However, there is insufficient reference in available literature about its manometric and point criteria for fecal incontinence [1].

Fig. 1. *Picture of the device for anorectal manometry Peritron (Cardio Design Pty Ltd, Oakleigh, Victoria, Australia)*

MATERIALS AND METHODS

The retrospective study was based on the results of ARM using the Peritron manometer (Cardio Design Pty Ltd, Australia) in 1200 patients with various proctological diseases, The patients were examined and treated at the "Outpatient Proctology Center" of the Railway Clinical Hospital (Rostov-on-Don, Russia) from 2015 to 2020. Their age varied from 30 to 70 years (average age — 47,4±13,6 years). There were 623 women (51,9%), and 577 men (48,1%). Most patients were in the age group from 31 to 40 years (29,3%) and 41 to 50 years (35,4%), which corresponds to the most active, healthy and productive period of life. All patients were divided into 4 groups in accordance with the clinical classification of Fecal Incontinence (FI), developed at Ryzhikh National Medical Research Center of Coloproctology (Moscow, Russia) and the results of measurements. The Cleveland Fecal Incontinence Scale (Wexner) was used as a subjective assessment and the results of ARM using the Peritron were used as an objective assessment.

When clinically examining the muscular bundle of the external rectal sphincter and pelvic floor in patients, we used the Peritron with a built-in biofeedback function (BFF), which can thus be used as a simulator for therapy Biofeedback. Biofeedback therapy or biofeedback rehabilitation is a completely harmless and effective method to restore proper functioning of the pelvic floor muscles by teaching the patient to relax or contract the anal sphincter/pelvic floor muscles when it is necessary. In particular, during a postoperative rehabilitation course for the prevention and treatment of incontinence, the device allows controlling the contractions of the pelvic floor muscles in order to restore or improve existing physiological skills and to assess the strength of muscle contraction further on.

The Peritron sphincterometer consists of three parts: two sensors — anal and vaginal (pneumatic) sensors, which are alternately connected using a flexible hose to a device with a monitor, which dis-

plays data on the contraction force of the pelvic floor muscles: basal tone/resting pressure and tone/pressure when straining. The measurement units can be manually selected on the device from the list of proposed parameters. We used 'centimeter of water column' (cmH_2O) as a measurement unit in our study.

ARM technique using Peritron: In the supine position on a gynecological chair with the lower body exposed, the rectal probe was inserted 6–8 cm into the anal canal. Next, the patient was asked to relax as much as possible. According to the indications on the device, data on the basal rest tone/pressure of the external sphincter of the rectum were measured. The values were reset to zero by pressing the corresponding button on the device and without changing the position of the rectal sensor, then the patient was asked to squeeze the rectal sphincter as much as possible using the phrase: *Imagine you want to hold gas/feces. Try to squeeze the anal sphincter as tightly as possible or strain so that they do not come out*. The values were measured for 5 attempts with a 10-second relaxation period, the average value is chosen — tone/pressure upon straining. To assess the force of contraction of the external sphincter of the rectum, the obtained data of the sphincterometer were correlated with the assessment on the Wexner scale (in points ranging from 0 to 20) (Table 1).

RESULTS

To determine the reference manometric criteria of various degrees of anal sphincter insufficiency (fecal incontinence) according to the ARM data using the Peritron, we analyzed the following criteria: mean resting pressure; maximum contraction pressure; average contraction pressure; volitional contraction gradient. All results were split by gender: for men and for women. The results are presented in tables 2 and 3.

Statistical data processing was carried out using the Statistica 10.0 program. (StatSoft, USA). The calculation of the mean value, standard deviation, minimum and maximum values (Me ± SD, min–max) was performed. The analysis used descriptive statistics methods (number of patients, mean, confidence interval, minimum and maximum values, standard deviation, standard error).

When comparing groups of patients, we used nonparametric statistical methods, for example, U — Mann–Whitney test (nonparametric alternative to t-test for independent samples).

A One-way analysis of variance performed according to gender revealed significant differences for all variables. Therefore an additional comparative analysis of variables using Student's t-test was performed for patients with varying degrees of FI . As a result, significant differences were revealed in all observed variables among all degrees: mean resting pressure, maximum contraction pressure, average contraction pressure, volitional contraction gradient, which made the rendering of reference intervals possible.

A subjective score assessment (Wexner scale) was used to analyze the manometric parameters obtained for all degrees of fecal incontinence. One-way analysis of variance revealed significant differences between various FI degrees according to the FI severity score results ($p<0,01$). Taking this into account, a comparative analysis of this variable in the observed groups was carried out using the Student's t-test. As a result, significant differences were revealed among all degrees of FI, which made rendering reference intervals possible. For men with first-degree FI on the Wexner scale, a value of ≤4.4 points was obtained. For second-degree FI, the indicator ranged from 4.4 to 10.5 points. In turn, for third-degree FI, the characteristic value was ≥10.5 points. For women with first-degree FI on the Wexner scale, a value of ≤6.7 points was obtained. For the second-degree FI, the indicator ranged from 6.8 to 10.9 points. In turn, for third-degree FI, the characteristic value was ≥11.0 points.

CONCLUSION

Anorectal manometry using the Peritron equipment is an affordable, simple, reliable, and cost-effective method for determining the norm and different degrees of Fecal Incontinence (FI) at various stages of diagnosis, monitoring the efficiency of surgical treatment and rehabilitation therapy in proctological patients.

The identified manometric reference criteria (based on ARM data using the Peritron) in correlation with the score criteria for fecal incontinence according to the Wexner scale will allow a quantitative analysis of incontinence in patients, which will thus enable its widespread use for screening studies and correctly forming prescriptions for expensive and rare studies to assess the state of the muscular bundle of the pelvic floor, its obturator function with specialized equipment.

Economic affordability and the built-in biofeedback function of the Peritron 9600 sphincterometer also provides a unique opportunity to use it as a home simulator for rehabilitation therapy — biofeedback therapy — in patients with fecal incontinence.

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Table 2. *Manometric (the Peritron) and point (Wexner scale) parameters of various degrees of fecal incontinence (FI) in men*

Degree of FI	Manometric indicators (cmH_2O)				Wexner scale score (points)
	Mean resting pressure	Maximum contraction pressure	Average contraction pression	Volitional contraction gradient	
I	45,9–61,2	152,1–154,4	123,8–133,5	>107,9 (normal)	< 4,4
II	33,7–42,6	101,3–148,1	72,6–121,5	72,6–105,5	4,4–10,5
III	<33,6	<101,2	<72,5	<67,8	> 10,5

Table 3. *Manometric (the Peritron) and point (Wexner scale) parameters of various degrees of fecal incontinence (FI) in women*

Degree of FI	Manometric indicators (cmH_2O)				Wexner scale score (points)
	Mean resting pressure	Maximum contraction pressure	Average contraction pression	Volitional contraction gradient	
I	49,7–54,8	133,4–149,3	94,2–119,2	>100,8 (normal)	< 6,7
II	36,8–49,6	84,8–133,3	63–94,1	49,1–79,5	6,8–10,9
III	<36,7	<84,6	<62,8	<49,1	> 11,0

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CONTENT OF PRIMARY AND SECONDARY LIPID PEROXIDATION PRODUCTS IN SUBCELLULAR FRACTIONS OF CARDIOMYOCYTES DURING MYOCARDIAL INFARCTION IN RATS IN AN EXPERIMENT AND THEIR CORRECTION BY TRANSPLANTATION OF MESENCHYMAL STEM CELLS

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ABSTRACT — Experimental modeling of myocardial infarction in rats was carried out by ligation of the anterior intergastric artery after the first division. There were 3 groups of 20 animals each: control group I — to verify normal parameters, group II — a model of myocardial infarction, and group III — animals which, after modeling myocardial infarction, underwent transplantation of mesenchymal stem cells. The level of lipid peroxidation products — diene conjugates and malondialdehyde — was studied by spectrophotometry. Comparison of the content and their ratio in the cytoplasm and mitochondria of myocardiocytes was carried out. It turned out that transplantation of mesenchymal stem cells significantly levels the activation of lipid peroxidation processes in subcellular fractions of cardiomyocytes, which is accompanied by a decrease in the primary and secondary products of oxidative stress. The ratio of malondialdehyde to diene conjugates both in the cytoplasm and in the mitochondria of cardiomyocytes after transplantation returned to control values. This indicates the normalization of physiological processes with underlying ischemic heart damage. The results indicate the cytoprotective effect of mesenchymal stem cell transplantation and the preservation of a larger number of cell pools, compared with the control group of animals that did not receive any treatment.

KEYWORDS — myocardial infarction, stem cell transplantation, lipid peroxidation, mesenchymal stem cells, subcellular fractions of cardiomyocytes, diene conjugates, malondialdehyde.

INTRODUCTION

Despite significant research devoted to myocardial infarction (MI) and its treatment, a number of pathophysiological questions remain open and require further study [1]. Existing therapies reduce early mortality, prevent additional damage to the heart muscle, and reduce the risk of further heart attacks. However, there is a need for a treatment to improve the infarction area by replacing damaged cells after MI. [2, 3]. The irreversible loss of cardiomyocytes and the formation of scar tissue after myocardial infarction leads to progressive impairment of cardiac function. Thus, cardiac repair (replacement, repair and regeneration) is essential for cardiac function after myocardial infarction. It is a well-known factor that the earlier the treatment of myocardial infarction is started, the more effective the therapy is. However, for a number of reasons, it is not always possible to make a diagnosis on time and, accordingly, begin treatment [4, 5]. Recently, in addition to standard methods of treatment, transplantation of various cell fractions and cytokine cocktails for myocardial infarction has been increasingly used. Mesenchymal stem cells (MSCs) are obtained from a wide variety of sources and are easy to isolate and culture. MSCs are capable of amplification and self-renewal in vitro, have low immunogenicity and immunomodulatory properties, and under certain conditions MSCs can differentiate into various cells. In the cardiovascular system, MSCs can protect the myocardium by reducing inflammation, promoting myocardial cell differentiation around infarction areas and angiogenesis, increasing resistance to apoptosis and suppressing fibrosis, which is ideal for cardiovascular recovery.

Therefore, stem cell regeneration of heart tissue may be an effective treatment option. Recently, they are increasingly inclined towards myocardial regeneration using stem cells [6, 7]. The potential benefits and the possibility of improving cardiac function with stem cell therapy require further research, which was the reason for this study.

The aim of the study is to study the dynamics of lipid peroxidation (LPO) products in rats with myocardial infarction and the effect of transplantation of MSCs on them.

MATERIALS AND RESEARCH METHODS

An experimental study was carried out on Wistar-Kayoto rats weighing 200–220 g. Research at all stages was carried out in a certified laboratory. The experiment was carried out in compliance with the "European Convention for the protection of vertebrate animals used for experiments or other scientific purposes" [Directive 2010/63 / EU]. We formed 3 groups of 20 animals each: control group I — to verify normal parameters, group II — a model of myocardial infarction, and group III — animals that received MSC transplantation after modeling myocardial infarction. Thoracotomy and ligation of the anterior intergastric artery after the first division were performed under intra-abdominal anesthesia with calypsol + xylazine, thus forming a model of myocardial infarction. In normal animals, after MI modeling, hearts and myocardiocytes were removed after 60 minutes and 1 day under anesthesia.

The method for determining the content of diene conjugates (DC) is based on the intense absorption of conjugated diene structures of lipid hydroperoxides in the 232 nm. Determination of the content of malonic dialdehyde (MDA) in lipid systems was studied by its interaction with 2-thiobarbituric acid leading to the formation of a chromogen with a maximum absorption in the red region of the visible spectrum at a wavelength of 532 nm. The measurements were carried out on an SF-56 spectrophotometer (Russia). Comparison of the content and their ratio in the cytoplasm and mitochondria of myocardiocytes was carried out.

Statistical data processing was carried out using Statistica 8.0 software packages. The statistical significance of the differences was determined using the Mann–Whitney rank test. The critical level of significance when testing statistical hypotheses was taken equal to $p < 0.05$.

RESULTS AND THEIR DISCUSSION

After studying the effect of MSC transplantation on the total level of peroxidation in the blood plasma after MI, it seemed important to assess the intensity of LPO directly in cardiomyocytes at the level of subcellular fractions. In experimental animals, the content of primary and secondary lipid peroxidation products in subcellular fractions of rat cardiomyocytes was studied 1 hour and a day after MI. The content of MDA and DC was determined in the cytoplasmic and mitochondrial fractions of cardiomyocytes.

Myocardial ischemia naturally caused an increase in the content of MDA and DC (LPO products). 1 hour after MI, the most significant increase in the content of primary DC products was 5.8 and 3.3 times in the cytoplasmic and mitochondrial fractions, respectively. The concentration of MDA after an hour of ischemia also increased: in the cytoplasmic fraction of cardiomyocytes, an increase of 4.6 times was noted, and in the mitochondral fraction — 2.7 times compared with the control (Table 1).

From the results obtained, it follows that that under conditions of ischemia in the subcellular fractions of cardiomyocytes, the processes of peroxidation after MI are more pronounced than the total indices of blood plasma. Both DC and MDA accumulate, which indicates the most significant peroxide modification of cardiomyocytes. A decrease in the ratio of MDA and DC is characteristic, reflecting the depth of LPO development in myocardial tissue in normal conditions and in MI.

When studying the parameters of primary and secondary lipid peroxidation in subcellular fractions of rat cardiomyocytes 1 day after MI, a slight decrease in the level of DC in the cytoplasm and in mitochondria was found. The total indicator — the MDA / DC ratio in the cytoplasm decreased by only 8% compared to the level of peroxidation 60 minutes after MI (Table 2).

Thus, in the group with MI, the concentration of DC in the cytoplasm is 7.13 ± 0.24 mol/g of wet weight -10'5, and in the control one — 1.39 ± 0.07 mol/g of wet weight — 10^5 at $p < 0.05$. In the group of animals with MSC transplantation, a decrease in the level of DC in the cytoplasm was established to 2.42 ± 0.08 mol/g of wet weight — 10^5, which is significantly lower than in the MI group, but nevertheless higher than in the control one.

A similar picture is observed when studying the MDA level in the cytoplasm of cardiomyocytes. So, in the control group, the MDA level is 0.146 ± 0.006 mol/g of wet weight — 10^5, and with MI this indicator decreased compared to the group of animals 60 minutes after the MI, but, nevertheless, remained much higher than the control one — 0.533 ± 0.027 mol/g of wet weight — 10^5 at $p < 0.05$. The same tendency was observed in the MI + MSC transplantation group: the MDA level was slightly higher than in the control one — 0.188 ± 0.014 mol/g of wet weight — 10^5 at $p < 0.05$, but much lower than in the group with MI.It is especially significant that the ratio coefficient (MDA / DC), which significantly decreased with MI to 0.072 ± 0.004 mol/g of wet weight — 10^5 at $p < 0.05$, did not differ from the control one in the group MI + MSC transplantation at $p > 0.05$ and amounted to 0.095 ± 0.005 mol/g of wet weight —10^5.

In the study of the content of primary and secondary LPO products in the mitochondrial fraction of cardiomyocytes, a similar picture was

Table 1. *Content of primary and secondary LPO products in subcellular fractions of rat cardiomyocytes in normal conditions and 60 minutes after MI*

Fraction of myocardium	Conditions of the experiment	DC, mol / g of wet weight -10^5	MDA, mol / g of wet weight -10^5	MDA / DC ratio
	Control	1,39 ±0,07	0,146 ±0,006	0,102 ±0,003
Cytoplasm	MI 60 min.	8,26 ±0,34*	0,652 ± 0,031*	0,081 ± 0,005*
	Control	0,43 ± 0,03	0,047 ± 0,002	0,106 ±0,004
Mitochondria	MI 60 min.	1,48 ±0,04*	0,14 ±0,003*	0,089 ±0,004*

Note: *the difference between the norm and the group with MI is significant (p <0.05) — *.*

Table 2. *Content of primary and secondary lipid peroxidation products in subcellular fractions of rat cardiomyocytes 1 day after MI and in the MI + MSC group*

Fraction of myocardium	Group	DC, mol / g of wet weight -10'5	MDA, mol / g of wet weight -10'5	MDA / DC ratio
	Control n= 20	1,39 ±0,07	0,146 ±0,006	0,102 ±0,003
Cytoplasm	MI n=20	7,13 ±0,24*	0,533 ± 0,027*	0,072 ± 0,004*
	MI+MSC n=20	2,42±0,08**,***	0,188±0,014**,***	0,095±0,005**,***
	Control n=20	0,43 ± 0,03	0,047 ± 0,002	0,106 ±0,004
Mitochondria	MI n=20	1,21 ±0,03*	0,12 ±0,011*	0,089 ±0,004*
	MI+MSC n=20	0,61±0,04**,***	0,057±0,003**,***	0,094±0,005**,***

Note: *the difference between the norm and the group with MI is significant (p <0.05) — *;*
*the difference between the groups of MI and MI with MSC transplantation is significant (p <0.05) — **;*
*the difference between the normal and MI + MSC groups is significant (p <0.05) — ***.*

observed. Thus, 1 day after modeling MI, the level of DC in comparison with the norm decreased slightly (1.21 ± 0.03 mol/g of wet weight — 10^5 at p <0.05). In the group MI + MSC transplantation, it was 0.61 ± 0.04 mol/g, which was significantly higher than in healthy animals, but lower than in the group of rats with MI.

The content of MDA in mitochondria of cardiomyocytes in the group of animals with MI remained at a high level of 0.12 ± 0.011 mol/g of wet weight — 10^5 at p <0.05 compared to the control one; and in the group of MI + transplantation MSC it was 0.057 ± 0.003 mol/g of wet weight — 10^5, which is lower than in the group with MI, but higher than in healthy animals at p <0.05. An interesting fact is that the integral MDA/DC index decreased in the group with MI to 0.089 ± 0.004 at p <0.05, and in the MI + MSC transplantation group it did not significantly differ from the norm and amounted to 0.094 ± 0.005 at p <0.05.

CONCLUSION

Thus, MSC transplantation significantly reduces the activation of LPO processes in subcellular fractions of cardiomyocytes, which is accompanied by a decrease in the primary and secondary LPO products. It should be emphasized that the MDA/DC index both in the cytoplasm and in the mitochondria of the cardiomyocyte after MSC transplantation returned to the control values, which indicates the normalization of LPO processes during MSC transplantation with underlying ischemic heart damage. The obtained results indicate the cytoprotective effect of MSCs due to various mechanisms that lead to the preservation of a larger number of cell pools, compared with the group of animals that did not receive any treatment.

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EARLY SURGICAL TREATMENT OF ACUTE CORONARY SYNDROME IN PATIENTS WITH ST-SEGMENT ELEVATION (AT < H24), ARRHYTHMIC COMPLICATIONS AND SEVERE MITRAL REGURGITATION

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ABSTRACT — Treatment outcomes of acute coronary syndrome in 108 patients with ST-segment elevation (after more than 24 hours) complicated by arrhythmias (atrial fibrillation and atrial flutter) and subsequently developed severe (degrees 2 and 3) mitral insufficiency were analyzed. We elaborated an algorithm for the sequence of interventions. All the patients had coronary angiography and stenting of the symptom-dependent artery during the first 2 days after the admission. Depending on the localization of the left ventricular myocardial infarction zone, the patients were divided into 3 groups: 68 (63%) patients with anterior wall and apex infarction; 23 (21.3%) patients with posterior wall infarction; 17 (15.7%) patients with lower wall infarction. It was revealed that patients, who had undergone revascularization in the early stages, had a statistically significant increase in the main indicators of myocardial contractility, in contrast to patients receiving only conservative therapy. In the observation group, sinus rhythm was restored in all patients 1–4 days after hospitalization. 12 patients underwent emergency electrical cardioversion on the day of admission. In 31 patients, sinus rhythm was restored within the first 24 hours during therapy prior to revascularization. In 39 patients, sinus rhythm was restored during therapy on days 3–5 after primary revascularization. In 26 patients, sinus rhythm was restored 2–4 days after complete revascularization. Based on the study, several conclusions can be drawn: in conditions of heart failure, revascularization is best to perform at the earliest possible stage. The optimal values of vital functions for performing revascularization can be considered: systolic blood pressure ≥ 80 mm Hg; heart rate ≤ 110 beats per minute. In primary surgical treatment, it is most optimal to revascularize only the symptom-dependent artery. Re-intervention for complete revascularization is most effective and safe 10–14 days after the initial intervention. The use of emergency electrical cardioversion in those patients in the preoperative period is fully justified, despite the existing risks. Application of our algorithm helps to restore sinus rhythm, reduce the degree of mitral regurgitation and reduce mortality.

KEYWORDS — acute coronary syndrome, ST segment elevation, atrial fibrillation, atrial flutter, coronary angiography, coronary stent placement, left ventricular remodeling.

INTRODUCTION

Patients with acute coronary syndrome with ST-segment elevation (after more than 24 hours), complicated by rhythm disturbances (atrial fibrillation and atrial flutter) and subsequently developed severe (degrees 2 and 3) mitral insufficiency are referred to as critically ill patients [1, 2, 3]. With myocardial infarction over 24 hours, the need for coronary angiography and revascularization in an urgent manner remains controversial, and concomitant tachyarrhythmias, decreased left ventricular ejection fraction and severe mitral regurgitation are often perceived as relative contraindications to the aforementioned procedures. It should be noted that such patients are often admitted in an extremely serious or critical condition, which requires prolonged infusion of adrenomimetic drugs, which in turn lead to an increase in heart rate and aggravation of heart failure symptoms [5, 6]. The use of intra-aortic balloon counterpulsation to stabilize the condition of such patients is often ineffective because it does not coincide with the systole-diastolic mechanism [7].

The conservative treatment has low effectiveness in such patients and often results in death. The effectiveness of the use of systemic thrombolysis is also questionable. The possibility of using antiarrhythmic drugs to reduce heart rate or restore sinus rhythm is limited due to severe hypotension. The timing and sequence of invasive procedures is also the subject of much debate. The prevalence of endovascular and cardiac surgeons in the clinic determines the tactics of surgical treatment [8, 9, 10]. The use of radiofrequency ablation methods as the first stage is not indicated for such patients [11].

The reference literature describes cases of successful treatment of such patients, but with infarction within 6–8 hours [12]. However, in the case when optimal time for revascularization has been missed,

the issue of treatment tactics for such patients is very relevant, especially while patient can be transported [13, 14].

The purpose of this article is to analyze the results of treatment of patients with acute coronary syndrome with ST-segment elevation (after more than 24 hours), complicated by tachyarrhythmias (atrial fibrillation and atrial flutter) and subsequently developed severe (degrees 2 and 3) mitral insufficiency in our clinic in the period of 2005–2021 and the creation of an algorithm for the sequence of interventions.

MATERIALS AND METHODS

This article is based on the treatment outcomes for acute coronary syndrome in 108 patients with ST segment elevation (after more than 24 hours) complicated by arrhythmias (atrial fibrillation and flutter) and subsequently developed severe (degrees 2 and 3) mitral insufficiency. The study took place at our clinic (Georgievsky Crimean State Medical Academy, Simferopol) in the period from 2005 to 2021. The average age of the patients was 63 ± 2.4 years (from 49 to 78 years). There were 74 (68.5%) men and 34 (31.5%) women. All of the above patients were admitted to our clinic presumably 24–36 hours after the onset of left ventricular myocardial infarction. ECG of all patients showed atrial fibrillation (89 patients — 82.4%) or atrial flutter (19 patients — 17.6%) with a heart rate of 120 to 160 bpm (average heart rate was 135 ± 12 bpm). Systolic blood pressure was from 75 to 100 mm Hg (average systolic blood pressure 87 ± 6 mm Hg). Average SpO2 was 80 ± 4%. All patients were admitted to the clinic in an extremely serious condition with symptoms of incipient cardiogenic shock.

All the patients underwent coronary angiography and stenting of the symptom-dependent artery during the first 2 days after admission to the clinic. Based on angiography data, single-vessel coronary artery disease was observed in 43 (39.8%) patients (thrombotic occlusion of 1 or 2 portions of LAD LCA — 17 patients; thrombotic occlusion of 1 or 2 portions of LCX — 12 patients; thrombotic occlusion 1 or 2 portions of RCA — 17 patients). Two-vessel disease was observed in 45 (41.7%) patients: LAD LCA + LCX - 21 patients, LAD LCA + RCA - 14 patients, LCX + RCA — 10 patients. Three-vessel disease (LAD + CX + RCA) was observed in 20 (18.5%) patients. The infarction-associated artery was considered LAD in 68 (63%) patients, CX in 23 (21.3%) patients and RCA in 17 (15.7%) patients respectively. Urgent coronary angiography was performed in 24 (22.2%) patients due to recurrent pain syndrome. On the day of admission, all patients underwent echocardiography which data are presented in Table 1.

Depending on the localization of the left ventricular myocardial infarction zone, the patients were divided into 3 groups. 68 (63%) patients had anterior wall and LV apex infarction; 23 (21.3%) patients had LV posterior wall infarction; 17 (15.7%) patients had LV inferior wall infarction. None of the patients had undergone systemic thrombolysis at the prehospital stage due to the late decision of patients to seek medical help.

For a more objective analysis of treatment results, 58 patients were assigned to a control group (mean age — 72 ± 6.2 years). They underwent early (≤48 hours) revascularization after hospitalization and control coronary angiography 14–21 days after hospitalization. These patients had been admitted to the clinic 24–48 hours after the alleged myocardial infarction of the anterior LV wall with concomitant atrial fibrillation on the ECG (heart rate was from 115 to 145 beats per minute (mean heart rate was 128 ± 14 bpm) and severe mitral regurgitation. Systolic blood pressure was from 85 to 110 mm Hg (mean systolic blood pressure was 93 ± 8 mm Hg). Mean SpO_2 was 88 ± 3%. Due to the absence of signs of cardiogenic shock and pulmonary edema, it was decided to continue the conservative therapy for 2–3 weeks (coronary angiography was declined by the patients).

The study involved standard research methods (instrumental, clinical and laboratory). The results of the patients' treatment were analyzed within 6 months. The study traced the change in the degree of mitral valve insufficiency, change in the pumping function of the left ventricle by measuring LVEF, end-systolic dimension (ESD), end-diastolic dimension (EDD), end-diastolic volume (EDV), end-systolic volume (ESV) before and after surgical correction of coronary arteries stenosis.

The obtained results were statistically processed using the Statistica 10.0 software (StatSoft Inc., USA) by determining the median (Me) and interquartile range (Q1–Q3). The statistical significance of differences between groups was assessed using the nonparametric Mann-Whitney test. Values were considered significant at $p < 0.05$.

RESULTS

All patients in the observation group underwent coronary angiography and stenting within the first 48 hours after admission to the clinic. 24 patients (22%) underwent coronary angiography and subsequent stenting within 10 minutes after the admission. 82 patients (78%) had the intervention postponed (within 24-48 hours after admission) until their stabilization. Intra-aortic balloon counterpulsation (IABP) was placed in 43 patients (40.6%).

Table 1. *Patients' echocardiographic data*

	Statistical parameter	Patients with single–vessel coronary artery disease	Patients with two–vessel coronary artery disease	Patients with three–vessel coronary artery disease	Control group of patients
Number of patients		43	45	20	58
LVEF (%)	M±σ Me [Q1–Q3]	32,3±4,7 [27,3–38,7]	28,6±2,9 [24,3–32,7]	27,5±3,6 [23,3–32,4]	36,2±2,9 [32,5–41,4]
EDV (ml)	M±σ Me [Q1–Q3]	162± 22 [126–189]	173± 18 [148–195]	167± 23 [134–199]	165± 19 [146–188]
ESV (ml)	M±σ Me [Q1–Q3]	106± 13 [91–121]	124± 17 [102–143]	119± 14 [112–127]	102± 9 [89–119]
SV (ml)	M±σ Me [Q1–Q3]	49± 6 [41–59]	53± 8 [42–68]	47± 3,5 [41–53]	59± 3,5 [53–66]
EDD (sm)	M±σ Me [Q1–Q3]	5,77±0,27 [5,32–6,32]	5,62±0,27 [5,04–5,91]	5,59±0,2 [5,05–5,89]	5,43±0,31 [5,27–5,72]
ESD (sm)	M±σ Me [Q1–Q3]	4,28±0,15 [4,13–4,62]	4,31±0,15 [4,12–4,59]	4,4±0,13 [4,15–4,55]	4,5±0,19 [4,32–4,65]
Vena contracta on the MV (mm)	M±σ Me [Q1–Q3]	6,2±0,6 [4,9–6,9]	6,3±0,5 [5,2–7,1]	7,1±0,9 [4,9–8,2]	6,4±1,1 [5,1–7,9]
Aortic calcification, number of patients (%)		12 (28%)	25 (60%)	14 (70%)	32 (55%)

Abbreviations: *LVEF — left ventricular ejection fraction; EDV — end-diastolic volume of the left ventricle; end-systolic volume of the left ventricle; SV — stroke volume; EDD — end-diastolic dimension; ESD — end-systolic dimension; Vena contracta on the MV — narrowed vein on the mitral valve.*

We considered the optimal indicators of vital functions for CAG as follows: systolic blood pressure ≥ 80 mm Hg; heart rate ≤ 110 bpm. Based on coronary angiography data, all patients underwent stenting of the symptom-dependent artery at the first stage, and had complete revascularization at the second stage (after 10–12 days).

On the 5th day after the first stage of stenting, the patients underwent control echocardiography (Table 2). It was found that patients with mono-lesion had a statistically significant decrease in EDD (by 15.6%), ESD (by 16.1%), EDV (by 23.5%), and ESV (by 31.4%); that led to an increase in systolic function of LVEF (increase in ejection fraction by 31.3%), ($p < 0.05$). There was a significant decrease in the diameter of the regurgitation wave on the mitral valve to 3.8 ± 0.2 mm (decrease by 34.6%). Patients with two-vessel disease had a statistically significant decrease in EDD (by 12.1%), ESD (by 13.5%), EDV (by 17.2%), and ESV (by 23.3%); that led to an increase in systolic function of LVEF (increase in ejection fraction by 18.4%) ($p < 0.05$). There was a significant decrease in the diameter of the regurgitation wave on the mitral valve to 5.7 ± 0.2 mm (decrease by 17.4%). Patients with three-vessel disease had a statistically significant decrease in EDD (by 11.3%), ESD (by 17.2%), EDV (by 16.6%), and ESV (by 27.4%). This led to an increase in LVEF systolic function (increase in ejection fraction by 26.8%) ($p < 0.05$). There was a significant decrease in the diameter of the regurgitation wave on the mitral valve to 6.2 ± 0.1 mm (decrease by 34.6%).

It was revealed that patients, who had undergone revascularization in the early stages, had a statistically significant increase in the main indicators of myocardial contractility, in contrast to patients receiving only conservative therapy. Tab. 2.

In the observation group, sinus rhythm was restored in all patients 1–4 days after hospitalization. 12 patients underwent emergency electrical cardioversion on the day of admission. In 31 patients, sinus rhythm was restored within the first 24 hours during therapy prior to revascularization. In 39 patients, the sinus rhythm was restored during therapy, 3–5 days after the primary revascularization was performed. In 26 patients, sinus rhythm was restored 2–4 days after complete revascularization.

Table 2. *Dynamics of patients' echocardiography on day 5 after the first stage of stenting*

	Statistical parameter	Patients with single-vessel coronary artery disease	Patients with two-vessel coronary artery disease	Patients with three-vessel coronary artery disease	Patients who have not undergone revascularization
EDD, sm	M±σ Me [Q1-Q3] p	5,037±0,15 [4,82-5,11] 0,005	5,12±0,17 [5,04-5,21] 0,005	5,18±0,22 [5,05-5,29] 0,0001	5,41±0,29 [5,23-5,70] 2,1
ESD, sm	M±σ Me [Q1-Q3] p	4,03±0,12 [3,95-4,19] 0,005	4,01±0,15 [3,88-4,21] 0,005	4,12±0,11 [4,0-4,24] 0,005	4,48±0,18 [4,23-4,76] 1,7
ESV, ml	M±σ Me [Q1-Q3] p	72±13 [59-107] 0,0001	77±12 [62-97] 0,005	78±15 [61-94] 0,005	102± 14 [85-117] 1,2
EDV, ml	M±σ Me [Q1-Q3] p	146±15 [123-171] 0,005	138±18 [114-165] 0,0001	144±21 [122-169] 0,0001	163± 18 [139-185] 1,3
EF, %	M±σ Me [Q1-Q3] p	42,8±2,8 [36,4-57,3] 0,005	38,9±2,4 [34,2-57,1] 0,005	36,4±2,3 [36,2-54,4] 0,005	37,1±1,7 [33,2-42,1] 0,79

Note: *p <0.05 — the differences are statistically significant compared to the value on the day of hospitalization*

In the control group, the sinus rhythm was restored in 34 patients, and the others had a persistent arrhythmia. In 17 patients, the sinus rhythm was restored and maintained. 7 patients had a persistent atrial fibrillation.

As a result of the treatment, 7 patients from the group of operated patients and 17 patients from the control group died (within 7 days after hospitalization). In the observation group, 3 patients died within 48 hours after the first revascularization due to ongoing cardiogenic shock; 1 patient died as a result of acute mesenteric thrombosis on the 5th day after primary revascularization; 2 patients died as a result of acute rupture of LV myocardium and tamponade on the days 12 and 14 after hospitalization; 1 patient died on the 9th day after hospitalization as a result of recurrent myocardial infarction and ventricular fibrillation. As can be seen from these statistics, despite the initially higher rates of left ventricular contractility, mortality was significantly higher in the group of non-operated patients. At discharge, a decrease in the severity of acute heart failure and an improvement in well-being were noted in all patients clinically.

DISCUSSION

This category of patients initially belongs to the high-risk group. Until now, there is no unambiguous algorithm for providing care, including surgical treatment, based on the duration of myocardial infarction. Developed AF with severe mitral regurgitation are aggravating factors, which in some cases is a contraindication to revascularization. Vital indicators (blood pressure, heart rate and SpO_2) in which coronary angiography followed by revascularization are not definite constant and vary depending on the specific situation. The minimal values of vital functions that we used for revascularization showed high efficiency of treatment, especially in comparison with other treatment tactics. The need for early revascularization, despite the existing risks, turned out to be fully justified, which is confirmed by the above data.

CONCLUSION

1. Despite the duration of myocardial infarction more than 24 hours, in conditions of severe heart failure, it is best to perform revascularization at the earliest possible terms.
2. Consider the optimal values of vital functions for revascularization as follows: systolic blood pressure ≥ 80 mm Hg; Heart rate ≤ 110 beats per minute.
3. Despite the higher rates of contractility, the survival rate is lower in the group where only conservative treatment was used
4. In the group of operated patients, the frequency of restoration of sinus rhythm is higher than in the group of non-operated patients.

5. The use of emergency electrical cardioversion in such patients in the preoperative period is fully justified, despite the existing risks.
6. There is a statistically significant decrease in the degree of mitral regurgitation as a result of left ventricular remodeling due to revascularization and restoration of sinus rhythm.
7. In primary surgical treatment, it is best to revascularize only the symptom-dependent artery.
8. Reoperation for complete revascularization is most effective and safe 10–14 days after the initial intervention.

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GASTROESOPHAGEAL REFLUX DISEASE AFTER SLEEVE GASTROPLASTY IN CLINICAL PRACTICE: A LITERATURE REVIEW

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ABSTRACT — This article focuses on the most effective and reliable method of obesity treatment — bariatric surgery. Laparoscopic sleeve gastrectomy is one of the most popular methods, due to which patients eat less food, thanks to a decrease in stomach volume as well as some humoral mechanisms. The most common complication of laparoscopic sleeve gastrectomy is gastroesophageal reflux disease. In this article, the authors offer a literature review, which presents data on the etiology, pathogenetic mechanisms, as well as modern and advanced methods of diagnosis and treatment of gastroesophageal reflux disease after laparoscopic sleeve gastrectomy.

KEYWORDS — laparoscopic sleeve gastrectomy; morbid obesity; gastroesophageal reflux disease; endoscopic antireflux treatments for GERD; cruroplasty.

The increasing prevalence of obesity is associated with an increase in the incidence of the complications of this condition. The most common diseases associated with obesity are type 2 diabetes mellitus, arterial hypertension, pathology of the musculoskeletal system, sleep apnea syndrome, varicose veins of the lower extremities. There is also evidence of an increased risk of developing gastroesophageal reflux disease (GERD) in obese patients [1]. Conservative treatments (low calorie diet and exercise) have been used successfully for weight loss over a long period of time. However, despite initial weight loss, as a rule, of 5% to 10% in the first 6 months, most of the weight is recovered. Various drugs, like orlistat, lorcaserin and phentermine, can in some cases be an alternative to diet and exercise, but they have a significant risk of side effects (pulmonary hypertension, valvulopathy, etc.) without significant weight loss and long-term improvement in metabolic syndrome [2]. Bariatric surgery has proven to be worthwhile and is recommended for obese patients with BMI (body mass index) above 40 or above 35 with concomitant diseases (metabolic syndrome, type 2 diabetes mellitus, non-alcoholic fatty liver disease, hyperlipidemia and hypertension, sleep apnea) [3]. Currently, the following methods of surgical treatment of obesity are widely used: intragastric balloon placement; gastric banding; Roux-en-Y gastric bypass; biliopancreatic diversion (SADI); mini gastric bypass; laparoscopic sleeve gastrectomy [4].

Intragastric balloon placement and gastric banding have a large number of side effects with relatively low efficacy in terms of weight loss and lasting result. The main disadvantage of these techniques is the lack of neurohumoral changes, which can achieve a greater effect in the treatment of obesity and affect the complications of this disease. Complications of bypass surgery can be divided by the time of occurrence into early (occurring in the first 30 days after surgery) and distant. The most common early complications observed in patients are staple line failure and intra-abdominal bleeding. Distant complications include GERD, dumping syndrome, and gastric stump stenosis [5].

Laparoscopic sleeve gastrectomy (LSG) is gaining traction as a bariatric procedure with proven efficacy for weight loss and obesity-related diseases [6]. LSG is becoming more widespread due to its effectiveness in terms of combination of restrictive and hormonal effects [7]. The stomach after LSG has a significantly lower volume than normal, which can significantly reduce the amount of food consumed (and therefore calories). In addition, the fundus cells that produce ghrelin are removed, which leads to a decrease in appetite [6]. Some data published by Chopra et al. showed a high percentage of resolution of concomitant diseases: 84% for diabetes mellitus, 49.99% for hypertension, 90% for asthma, 90.74% for obstructive sleep apnea [8]. Another advantage of this operation is its relative technical simplicity.

Many authors believe that LSG also has a number of complications, the most common of which is gastroesophageal reflux disease. According to other data, the incidence of this pathology after performing LSG reaches 27%. However, it should be noted

that postoperative GERD has a multifactorial nature. Cases of persistent remission of GERD in 25% after LSG have also been described [9, 10]. GERD is a rather serious pathology that leads to a large number of complications: reflux esophagitis, esophageal strictures, peptic ulcers of the esophagus, metaplasia of the mucous membrane (Barrett's esophagus), adenocarcinoma. DuPree CE et al. analyzed the effect of LSG on patients with GERD and compared the results with gastric bypass surgery (GB). Of the 4832 patients who underwent LSG, 44.5% had pre-existing GERD. 84.1% of them persisted with GERD symptoms after LSG, and only 15.9% had resolution of GERD. In LSG without symptoms of GERD before surgery, 8.6% developed de novo GERD after surgery. In comparison, GB resolved GERD in the majority of patients (62.8%) within 6 months after surgery (P <0.001) [11]. Another study by Rebecchi F et al. in 71 patients showed that LSG improves symptoms and controls reflux in the majority of morbidly obese patients with preoperative GERD, but the cohort was small. DeMeester and total acidity (% pH <4) decreased from 39.5 ± 16.5 to 10.6 ± 5.8, P <0.001; % pH <4 from 10.2 ± 3.7 to 4.2 ± 2.6, P <0.001), and de novo symptoms of GERD were observed in 5.4% of patients [11, 12, 13].

The clinical picture and diagnosis of GERD after LSG has no significant features. The diagnosis of GERD is established on the basis of typical clinical manifestations (heartburn and belching), supported by the response to empiric therapy with proton pump inhibitors (PPIs) [14]. Presence of corresponding symptoms (dysphagia, weight loss, anemia), atypical manifestations (chest pain, laryngeal symptoms) or lack of response to empiric PPI therapy require endoscopic examination with biopsy [15]. In addition, manometry, 24-hour pH monitoring and pH impedancemetry can be used to confirm the diagnosis and exclude other pathologies.

It has been proven that obesity is an independent risk factor for GERD development associated with mechanical changes in the gastroesophageal junction due to excessive relaxation of the lower esophageal sphincter and, as a consequence, return of food into the esophagus [16]. It is also known that overweight patients have an increase in the gradient of intra-abdominal pressure and intragastric pressure during the inspiratory phase, which influences the occurrence of persistent reflux [17]. According to the authors Edoardo Savarino et al., in patients with morbid obesity, in 70% of cases, there is a deterioration in esophageal motility due to the failure of the esophageal-gastric sphincter [18], which also leads to the appearance of GERD and reflux esophagitis. This may be due to the presence of a hiatal hernia. Some studies have shown that in patients with pre-operative GERD, after LSG, symptoms intensified; and in patients without signs of GERD, in the preoperative period, the first symptoms (heartburn, belching) appeared after surgery [19]. Hanaa Dakour Aridi et al. report that 73% of patients undergoing LSG developed lower esophageal sphincter insufficiency due to resection of a part of the muscle fibers during the surgery. Also, during the formation of the gastric *sleeve* it may be excessively narrowed, intragastric pressure increases and, as a result, motility of the stomach and lower esophagus is impaired [20]. In this regard, some authors believe that a timely transition from LSG to Roux-en-Y gastric bypass is the best option when choosing a tactic for GERD control.

In order to prevent postoperative GERD in bariatric patients, careful selection of LSG candidates is recommended (contraindications are large hiatal hernia, severe esophagitis, or severe long-term symptoms of GERD) as well as careful adherence to the surgery technique in combination with posterior cruroplasty (simple or reinforced). This guarantees efficacy with an overall relapse rate of 10.7% over 5 years. According to Cristian Eugeniu Boru et al. [21], the outcomes of LSG combined with posterior cruroplasty were satisfactory in all patients. The general trend confirms that reinforced cruroplasty is highly effective in a specific subgroup of patients with a "moderate" hiatus defect (4 to 8 cm2), without the side effects or complications associated with the use of a fully absorbable synthetic mesh. At the same time, an acceptable rate (80%) of GERD symptom control has been achieved with regression in relation to short-term results, while no patient had a single case of Barrett's esophagus after 60 months on EGD. The selection of patients for LSG should be patient-specific, taking into account all individual characteristics. To date, there are publications on new antireflux surgeries and modifications in obesity surgery. For example, da Silva et al. describe Sleeve-Collis-Nissen Laparoscopic Gastroplasty (LSCNG), a relatively new, technically feasible operation with a low incidence of related complications [22]. However, further prospective studies are needed to assess the real impact of this and a range of other treatments on improving GERD symptoms.

In order to reduce the severity of symptoms and improve the quality of life, a lifestyle change is recommended at the first stage of treatment. However, it is important to note that most studies have shown insufficient effectiveness of lifestyle and dietary changes in GERD [23, 24]. It has been shown that the elevated position of the head end of the bed reduces the effect of acid on the esophageal mucosa and the speed of pas-

sage through the esophagus, followed by a decrease in GERD symptoms [25]. In addition, it is recommended to minimize or eliminate factors affecting the incidence of transient lower esophageal sphincter relaxation. These include smoking, excessive alcohol consumption, heavy dinner, nighttime snacks, and high levels of fat in the diet. In addition, all patients with GERD should avoid non-steroidal anti-inflammatory drugs (NSAIDs) because of their role in impairing the physiological mechanisms of mucosal defense.

Drug therapy for GERD aims to reduce symptoms and minimize mucosal damage from acid reflux. Although acid suppression is a successful strategy in the treatment of GERD, there appears to be no clear association between the severity of GERD and high gastric acid levels, with the exception of Zollinger-Ellison syndrome [26]. Major acid suppressants include proton pump inhibitors (PPIs) and H2 blockers. H2 blockers reduce gastric acid secretion by inhibiting mast cell histamine stimulation. PPIs reduce the amount of acid released from the parietal cells into the stomach lumen. H2 blockers have been shown to have some symptomatic benefits over placebo, but PPIs are the most effective in persons without contraindications [27]. There is no clear role of prokinetic agents, such as metoclopramide, in the treatment of GERD [28]. PPIs are the most effective class of antacids. They should be taken once or twice a day 30–60 minutes before meals. Most patients experience recurrence of symptoms after discontinuation of PPI treatment, therefore lifelong therapy is often required [28]. Recently, there has been increased concern about PPIs, which are thought to contribute to bone fractures, electrolyte deficiencies, infections (eg, Clostridium difficile pneumonia) and renal failure [29, 30]. Given the theoretical risk of side effect from PPI therapy, the minimum therapeutic dose required for maintenance therapy should be used and periodic breaks in therapy should be attempted. There is some evidence that the addition of an overnight H2 blocker may be beneficial in patients with GERD resistant to the twice daily PPI dosage [31]. In refractory cases, other disorders should be considered, in particular: eosinophilic esophagitis, drug esophagitis, delayed gastric emptying, duodenogastric (biliary) reflux, irritable bowel syndrome, psychological disorders, achalasia and Zollinger-Ellison syndrome [32].

Surgical treatment of GERD includes minimally invasive endoscopic techniques as well as traditional surgical techniques. However, given the structural features of the gastric "sleeve" formed in LSG, Nissen fundoplication is impossible in such patients. Thus, gastric bypass surgery is a radical method of surgical treatment for GERD.

It should be noted that endoscopic antireflux therapies for GERD are still under development and most of published research focuses on symptom relief in the short term. Endoscopic treatments have become possible treatment options for people with GERD refractory to drug therapy. These methods include the following groups of interventions: endoluminal suture or plastic surgery of the gastroesophageal junction; radio pulse therapy of the lower esophageal sphincter (Stretta®); injection or implantation of biopolymers into the gastroesophageal junction [33]. More recently, two meta-analyzes have been described in the literature, one of which shows an overall increase in the effectiveness of transoral non-surgical fundoplication (TIF) performed with the EsophyX® device, compared to patients who did not undergo TIF [34]. The second study analyzes the Stretta procedure, showing no significant changes in physiological parameters (time at which the pH is less than 4, compression of the lower esophageal sphincter at rest, refusal of PPI drug therapy, and improvement in the quality of life according to GERD-HRQL) compared with sham therapy [35]. The main outcome of the study is to measure the overall efficacy of endoscopic treatment versus other treatments (PPIs or laparoscopic antireflux interventions) or sham treatments for chronic GERD. In 2018, a meta-analysis was published comparing the effectiveness of endoscopic treatment with pharmacological (PPI) treatment [36]. Analysis of the results of the treatment of 320 patients in 4 studies showed a greater efficacy of the endoscopic treatment (Stretta, exposure of the lower esophageal sphincter and cardiac stomach to radiofrequency energy; TIF2, transoral non-surgical fundoplication) compared with the control group. The endoscopic treatment was effective in treating chronic GERD in 69% of patients compared with 37% of patients treated with PPIs or sham + PPIs. In the analysis of individual subgroups, there was a statistically significant difference in favor of the endoscopic procedures at 6 months, but this difference was absent in the studies performed at 12 months [37]. These data suggest that these techniques have a temporary effect.

According to a study by Yves Borbély et al., electrical stimulation of the lower esophageal sphincter (LES-ES) in post-LSG patients with symptomatic PPI-resistant GERD results in a sustained reduction in symptoms and acid exposure to the esophagus in most patients. These data are preliminary. A larger sample size with longer follow-up is needed to confirm the results. The author states that longer follow-up may result in an even higher rate as the maximum effect is expected after 9 months, but the median of objective follow-up in this study was 6 months. While preserving anatomy after LSG, it offers a valid option

for patients who are unable or unwilling to undergo RYGB [38].

According to a study by Ryan C. Broderick et al. [39], placement of the LINX magnetic device is a safe and effective treatment for refractory GERD after bariatric surgery. This can relieve symptoms and eliminate the need for high-dose medical treatment or the transition to a more complex procedure. Magnetic dilation of the lower esophageal sphincter may be another surgeon's choice for managing reflux after bariatric surgery in some patients. According to the study, two patients developed complications requiring endoscopic dilation after LINX placement. 100% of patients reported overall satisfaction after the procedure.

Based on a comprehensive analysis of the literature data, the importance of early diagnosis and initiation of treatment should be emphasized in order to avoid the development of a wide range of complications. This requires close observation of patients in the postoperative period. The analysis of the literature on gastroesophageal reflux disease shows that the pathology is quite common in patients who have undergone laparoscopic sleeve gastrectomy. GERD as a complication of bariatric surgery requires the search for pathognomic symptoms and reliable examination methods, the study of possible causes of the development of the disease, as well as the selection of effective therapy.

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ADVANCES IN THE NEUROSURGICAL AND COMBINED TREATMENT OF PATIENTS WITH ACROMEGALY

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ABSTRACT — Aim. The study was carried out to identify and analyze the factors of a positive outcome of surgical and radiation treatment of acromegaly.
Methods. The work was performed on clinical material and summarizes treatment results of 256 patients (90 males and 166 females). 86% of patients underwent surgical treatment, 14% of patients received radiation therapy during 2002–2018. Significance of factors of a positive outcome of treatment was carried out using the RStudio program.
Results. The results of the study revealed that:
1. Significant factors ($p < 0.05$) of a positive outcome of surgical treatment of acromegaly are somatostatin analogs (SSA) therapy before surgery, a small tumor size (microadenomas), and the absence of extrasellar tumor spread. 2. The most significant factor in achieving remission of acromegaly after non-radical adenomectomy is postoperative therapy with SSA ($p < 0.05$). 3. Aggressive pituitary tumors invading surrounding structures, high baseline IGF-1 levels, unfavorable histological findings, macroadenomas, growth hormone levels above 10 μg/L before therapy, and extrasellar tumor spread were associated with less favorable outcomes of acromegaly radiation therapy (RT) ($p < 0.05$). The most significant factor in achieving remission of acromegaly is SSA therapy after RT ($p < 0.05$).
Conclusion. Surgical treatment is the optimal primary treatment for acromegaly. Drug therapy with SSA is effective and the preferred treatment after non-radical surgery.

KEYWORDS — acromegaly, combined treatment, surgical treatment, radiation therapy, drug therapy.

INTRODUCTION

The delay in diagnosis of acromegaly is still significant (4.5–5 years), and the disease is usually confirmed in the fifth decade of life in economically active population; this leads to loss of productivity, social and financial implications, and long-term burdens on the health system. Despite the long history of the study of acromegaly and a wide arsenal of modern methods of treatment, a complete clinical and laboratory remission is relatively rare. Hence, in February 2019, only 32% of acromegalic patients in Russia had complete clinical and laboratory remission of the disease (normalization of Insulin-like growth factor I (IGF-I)) [1]. In this regard, the search for an effective strategy for managing patients with this disease becomes relevant. Therefore, we analyzed the factors of a positive outcome of surgical and radiation treatment of acromegaly.

METHODS

The study was conducted at "N.N. Burdenko National Medical Research Center of Neurosurgery" (Moscow, Russia)

The work was performed on clinical material and summarizes treatment results of 256 patients (90 males and 166 females). 86% of patients underwent surgical treatment, 14% of patients received radiation therapy during 2002–2018.

Statistical processing of the treatment results was carried out using the RStudio program. Logit, probit, and gommit models were used. We also used McFadden's coefficient of determination, Likelihood-ratio, Kolmogorov-Smirnov test, Hosmer-Lemeshow test, error distribution graphs, and Pearson's correlation coefficient.

Besides, we calculated the marginal effects. The Student's t-test was used to assess the statistical significance of differences between samples.

To assess the effectiveness of treatment, the Cortina criteria (normal level of IGF-1 and growth hormone level after oral glucose tolerance test less than 1 μg/L) were used.

The average age of the patients was 46.03 ± 0.71 years. Endosellar adenomas were observed in 60% of cases, endo-suprasellar adenomas in 11% of cases, and endo-laterosellar adenomas also in 11% of cases. Endo-supralaterosellar tumor was observed in 8% of cases, endo-infrasellar in 10% of cases. In addition, 27 patients were admitted with a diagnosis of the condition after removal of pituitary adenoma, and in 1 case infrasellar adenoma was observed. Only 38 patients received drug therapy (SSA) before the operation, 1 patient had previously received radiation therapy.

RESULTS

There was a positive trend in the level of growth hormone (GH) and IGF-1. We noted that in the group of patients with surgical treatment, the level of GH was higher than in the group of radiation therapy (34.69 ± 2.04 ng/ml versus 26.97 ± 6.47 ng/ml).

In 93% of cases, after surgery, there was an improvement, in 4% there were no changes, and in 1% there was deterioration, recovery, and death (Fig. 1).

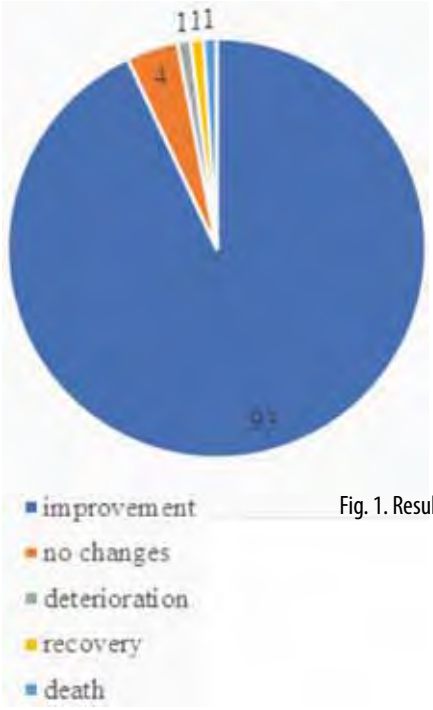

Fig. 1. Results of treatment, %

Before surgery, the level of GH was 34.69 ± 2.04 ng/ml, 12 months after surgery — 5.16 ± 0.35 ng/ml. We observed the normalization of the level of GH 12 months after the operation in 43% of patients.

In the group of patients with surgical treatment, the level of IGF-1 was lower than in the group of radiation therapy (619.08 ± 16.84 ng/ml versus 681.73 ± 27.22 ng/ml). Before surgery, the IGF-1 level was 619.08 ± 16.84 ng/ml, 12 months after surgery — 115.44 ± 4.48 ng/ml. We observed the normalization of the IGF-1 level 12 months after surgery in 41% of patients. A similar level of remission can be observed in other studies. So, Belaja J.E. et al. note that surgical treatment as the first line is 40.47% effective in achieving remission ($p < 0.01$) [1].

As a result of the study, we noted a statistically significant decrease in the levels of GH and IGF-1 against the background of surgical treatment 12 months after the operation ($p < 0.05$).

In addition, we noted a statistically significant decrease in the level of GH against the background of radiation therapy after 12 months ($p < 0.05$). Before treatment, the level of GH was 26.97 ± 6.47 ng/ml, 12 months after RT — 3.37 ± 0.82 ng/ml. We observed the normalization of the level of GH after 12 months in 38% of patients.

Before RT, the IGF-1 level was 681.73 ± 27.22 ng/ml, 12 months after RT — 130.39 ± 9.74 ng/ml. We observed the normalization of the IGF-1 level 12 months after RT in 43% of patients.

We also found a negative correlation between the level of GH before treatment and age ($r = -0.461$, $p < 0.01$). Consequently, at a younger age, higher levels of GH are noted. A similar connection is reported in the work by Molitvoslavova N.N. [2].

To optimize the treatment of acromegaly, we analyzed the key factors of the effectiveness of combination therapy. The results showed that the most significant factor was postoperative SSA therapy ($p < 0.05$). In addition, preoperative SSA therapy, tumor size, and extrasellar spread are significant factors.

DISCUSSION

Similar results were obtained by Carlsen S. M. et al. [3]. 62 patients took part in their study. The patients were divided into 2 groups: a group without pretreatment ($n = 30$) and a group with pretreatment with octreotide ($n = 32$) at a dose of 20 mg intramuscularly every 28 days for 6 months before transsphenoidal surgery. The results were assessed 3 months after the operation, mainly in terms of IGF-1 levels. As a result, 45% of pretreated patients versus 23% of patients undergoing direct surgery were cured ($p = 0.11$). At the same time, 20% of patients with microadenomas who received pretreatment were cured versus 60% using direct surgery ($p = 0.52$). 50% of pretreated patients with macroadenomas were cured, and 16% of patients with macroadenomas underwent direct surgery ($p = 0.017$).

Thus, six months of preoperative octreotide treatment may improve the surgical cure rate in patients with macroadenomas.

Fougner S. L. et al. [4] in their study also divided patients with acromegaly into 2 groups: the direct surgery group ($n = 30$) and the group with pretreatment with octreotide LAR for 6 months ($n = 32$). The analysis of the treatment results was carried out 1 and 5 years after the operation. The criteria for cure were normal IGF-1 levels and normal IGF-1 levels on an oral glucose tolerance test. 38% of macroadenomas were cured in the pretreatment group with octreotide versus 24% in the direct surgery group 1 year after surgery ($p = 0.27$) and 41% versus 27% of macroadenomas, respectively, 5 years after surgery ($p = 0.34$).

In addition, the results of our study are supported by two other prospective randomized studies showing the advantage of preoperative SSA treatment by doubling the remission rate and higher remission for tumors with invasion of the cavernous sinus [5, 6].

We also noted a statistically significant decrease in GH and IGF-1 levels 12 months after RT ($p < 0.05$). We found that the percentage of patients in remission usually increases with time after RT. For example, in the work of Powell J. S. et al. [7], 9 out of 13 patients (69.2%) observed at least 6 years after radiation therapy had normal IGF-1 levels without drug therapy. In 26 patients, IGF-1 levels decreased from 268 ± 32% (from the upper limit of normal) to 122 ± 12% after radiation therapy ($p < 0.001$).

The frequency of remission after different forms of radiation therapy probably differs [7], but the number of patients in each group and the number of follow-up periods in our study were very different. Therefore, we did not get a statistically significant difference between the groups.

In the Powell J. S. et al. [7] study, IGF-1 normalization was achieved in 14 out of 32 (43.7%) patients after remote radiation therapy (the average follow-up period was 5.6 years), in 3 out of 6 (50%) after stereotactic radiosurgical interventions (the average follow-up period was 2.9 years), in none of 4 patients after gamma knife LT (the average follow-up period was 1.2 years) and in none (1 patient) 3.6 years after proton beam radiotherapy.

In our study, hypopituitarism after RT was observed in 13% of patients. At the same time, we did not observe the development of new visual anomalies or secondary malignant neoplasms of the brain. In a study by Powell J. S. et al. [7] before receiving radiation therapy, 11 of 43 patients (25.6%) had a deficiency of at least 1 pituitary hormone, and 2 had panhypopituitarism. Pituitary hormone deficiency developed after radiation therapy in 13 of 41 patients (31.7%).

According to Minniti G., Scaringi C., Enrici R. M. [8], the main complication of radiation therapy is hypopituitarism, which is observed in 0–47% of patients. Pollock et al. [9] in their study noted that one-third of 39 patients with acromegaly had new hormone deficiencies after stereotactic radiosurgery (SRS), with a frequency of new hormone deficiencies in the anterior pituitary gland of 10% after 2 years and 33% after 5 years. In a series of 95 patients, hormone deficiency was observed in 5% 12 months after SRS but increased to more than 1/3 in patients with follow-up for more than 49 months. A similar incidence of hypopituitarism (20–40%) after 5 years has been observed in several other studies [10, 11]. Thus, we can assume that the incidence of this complication increases over time.

To optimize the treatment of acromegaly, we analyzed the key factors of the effectiveness of RT. As a result, it was revealed that the most significant factor in the positive outcome of RT is the administration of SSA after therapy ($p < 0.05$). Aggressive pituitary tumors with invasion of the surrounding structures, a high baseline IGF-1 level, an unfavorable histological picture, macroadenomas, a pretreatment GH level above 10 μg/L, and extrasellar tumor spread were associated with a less favorable RT outcome. For comparison, there are no similar data or research results on RT in the available literature.

CONCLUSION

The analysis made it possible to conclude that for patients with microadenomas of the pituitary gland and tumors of small sizes, with the absence of extrasellar growth, it is advisable to undergo surgical treatment, which has high efficiency in achieving remission of acromegaly. In agromegalic patients with pituitary macroadenomas and extrasellar spread, in most cases, combined treatment is necessary (therapy with SSA and/or radiation therapy). To increase the effectiveness of treatment in patients with macroadenomas and extrasellar tumor spread, it is advisable to use SSA therapy before and after the operation. Subsequent SSA therapy is required to improve the effectiveness of radiation therapy.

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CHRONIC PERIODONTITIS AND ENDOTHELIAL DYSFUNCTION IN PATIENTS WITH ARTERIAL HYPERTENSION

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ABSTRACT — The work offers a view at the data obtained through an examination of 120 patients with chronic generalized periodontitis (CP) and arterial hypertension (AH). The check-up included clinical examination, immunohistochemical, morphometric studies, as well as evaluation of the endothelium vasodilating function. CP in patients with hypertension features more significant changes in the quantitative density of gum cells positive to ET-1, endothelial NO-synthase, if compared to the CP values in patients with no background somatic pathology. Changes affecting vasoactive mediators in the gum are associated with a systemic inflammatory response and a violation of the endothelium vasodilating function. Periodontitis remission can be achieved with the therapy of the neurotransmitter imbalance.

KEYWORDS — periodontitis, arterial hypertension, NO-synthase, endothelin-1, endothelial dysfunction.

INTRODUCTION

Chronic periodontitis, which is the leading cause behind tooth loss, is often associated with cardiovascular health issues [4, 9]. Periodontitis and the circulatory system diseases share risk factors like age, smoking, diabetes and obesity [2, 12–15]. Chronic inflammatory processes, impaired immune response and oxidative stress are believed to be the basis of comorbidity between periodontitis and cardiovascular diseases [8, 10].

An important thing determining timely prevention of cardiovascular events is identifying vascular endothelial dysfunction. Endothelial dysfunction is a common pathway, which is taken by risk factors aiming to affect long-term atherogenesis processes [1]. The triggers of endothelial dysfunction include ischemia/hypoxia of tissues, elevated blood pressure, dyslipidemia, hyperinsulinemia, oxidative stress, chronic systemic inflammation and other factors [5]. Currently, the pathogenetic basis of endothelial dysfunction is viewed as residing in disturbed local production of nitric oxide (NO) and oxidative stress, which stimulate the synthesis of vasoconstrictors, lipid oxidation and damage to endotheliocyte membranes [11].

Endothelin (ET)-1, known as a vascular inflammation mediator and a vasoconstrictor, is expressed in the gum under the effect of pathogenic bacteria, cytokines, hypoxia, ischemia and mechanical stress [3]. The ET-1 levels in the gingival fluid and blood serum of patients with periodontitis exceed the similar indicator in healthy people and decrease following respective treatment [7].

The data on the relationship between periodontitis and endothelial dysfunction, which is to be found in the available literature, is scarce. There are single mentions claiming that severe periodontitis is associated with impaired endothelial function [6]. This study will help expand the understanding of the mechanisms behind the development of combined periodontal pathology and cardiovascular system, and identify the ways to promote prevention.

Aim: to study the vasoactive markers expression in the gum in patients with chronic periodontitis and arterial hypertension through the treatment dynamics, matching that against the specific features of the endothelial functions.

MATERIALS AND METHODS

The study included 120 patients with chronic periodontitis (CP) and arterial hypertension (AH) of Stage I–II (males — 62.5%; median age — 51.5±6.3). Sixty of the patients (50%) had mild CP, while another 60 (50%) featured moderate CP. As for a comparison group, there were 55 patients examined, who had mild and moderate CP with no somatic pathology (median age — 51.0±5.4); the control group included 25 basically healthy individuals. The examination was carried out prior to the treatment and then — 12 weeks into the treatment. As for the treatment, the following was

done: sanation and professional oral hygiene; antibacterial and anti-inflammatory treatment; surgical treatment, subject to respective indications. All patients received antihypertension therapy, with 93.3% of the patients arriving at the target blood pressure values.

The material for the morphological study was based on a biopsy of the gingival mucosa at the interdental papilla. The verification of the gum cells expression relied on the immunohistochemical method with primary monoclonal antibodies to endothelin-1 (ET-1) and to endothelial NO-synthase (eNOS). The immunohistochemical staining outcomes were evaluated through a morphometric study, with the relative expression area estimated.

The functional status of the endothelium was assessed through flow-induced endothelium-dependent vasodilation (EDV) of the right brachial artery with the standard ultrasound approach employed. The C-reactive protein blood levels were identified with the high-sensitivity method (hs-CRP).

All the participants were duly notified of the aims and of the study protocol, with respective written informed consents obtained from them prior to launching the study. The statistical processing of the outcomes results was performed with the IBM SPSS Statistical 21 software package employing the Mann-Whitney and Wilcoxon reliability criteria, the χ^2 Pearson criterion, and the Spearman correlation factor.

RESULTS AND DISCUSSION

The groups of patients with CP were comparable in terms of age and gender, while there were no statistically significant differences observed in the smoking rate, the body mass index and the glycemic level.

Patients with CP were found to have higher values of hs-CRP compared to practically healthy individuals (1.32±0.41 mg/l). The hs-CRP levels in patients with mild CP was 2.26±0.85 mg/l ($p<0.001$), whereas in patients with moderate CP these values were at 5.18±2.01 mg/l ($p<0.001$). Patients with CP and AH featured a more significant increase in hs-CRP compared to patients with just CP: 3.71±0.97 mg/l ($p<0.001$) for mild CP cases, and 8.52±4.18 mg/l ($p<0.001$) for patients with moderate CP.

The EDV index in the control group was 15.40±6.58%. Vasoreactivity in patients with mild CP remained unchanged (14.50±2.28%), while in patients with moderate CP it was significantly reduced compared to the control group members (13.07±2.26, $p<0.05$).

Patients with CP and AH revealed a more significant violation of the brachial artery EDV compared to patients with an isolated CP course, which was 12.35±2.14% and 10.93±1.60%, respectively, with mild and moderate CP ($p<0.05$). Disturbed EDV was observed in 41 (68.3%) patients with moderate hypertension and CP, and two times less often – in 21 (35%) patients with mild AH and CP ($\chi^2=30.06$, $p<0.0001$). Following the Spearman correlation analysis, a relationship was established between the PI and the EDV ($r = -0.44$, $p=0.005$); between the blood hs-CRP concentration and the EDV ($r = -0.48$, $p<0.001$).

Morphometric analysis showed that the expression of endothelium-associated markers, such as NO-synthase, ET-1 in patients with mild CP matched the control values, while patients with moderate CP showed a decrease in the expression of cells positive to NO-synthase (see Table 1).

Patients with CP combined with AH were found to feature hyperexpression in gum cells positive to ET-1, as well as a decrease in the expression of cells positive to NO-synthase, if compared to the control group, and patients with moderate CP featured a similar trend even compared to the values in patients suffering from CP with no AH. ET-1 plays an obviously important role in periodontal inflammation. The quantitative density of gum cells positive to ET-1 increased in pursuant to the severity of periodontitis, and correlated with the PI index ($r= 0.61$; $p<0.001$), the depth of periodontal pockets ($r= 0.48$; $p<0.001$).

The expression of endothelial markers, just like ET-1 and NO-synthase in the gingival vessels in case of CP and AH, must reflect some systemic change in the vascular wall, the evidence to that being the correlation of the number of gum cells immune-positive to endothelin-1 and NO-synthase with the EDV index ($r= -0.578$ $p<0.001$, Spearman; and 0.533, $p<0.001$, Spearman, respectively). The detected changes appear to be natural, since the combined effect of hemodynamic factors, as well as the systemic inflammatory response in case of moderate CP and AH can cause changes in the vasoregulatory function of the endothelium. Obviously, ET-1 can be viewed as one of the key mediators involved in the development of the mutual aggravation syndrome in periodontitis and AH.

The remission of periodontitis, when the majority of patients reach the target blood pressure, occurs against a positive dynamics of the gum morphofunctional status. After 12 weeks, the expression of gum cells positive to ET-1 and NO-synthase matched the indicators observed in basically healthy individuals (Table 1).

CONCLUSION

Patients with moderate CP without somatic pathology feature signs of endothelial dysfunction combined with a decrease in the expression of gum cells positive to NO-synthase. In case CP and AH are

Table 1. *Expression of gum cells immune-positive to endothelin-1 and nitric oxide synthesis in patients with periodontitis against AH, through the treatment dynamics*

Groups of patients	Value (expression area)	
	ET-1-positive cells (%)	eNO-synthase-positive cells (%)
Basically healthy, n =25	2.92±2.00	7.92±1.95
Patients with CP		
Patients with mild CP, n=25	3.20±1.65	6.88±2.24
Patients with moderate CP, n=30	3.37±1.77	6.30±2.23*
Patients with CP and AH prior to treatment		
Patients with mild CP combined with AH; n=60	6.08±2.35*&	5.94±2.31*
Patients with moderate CP combined with AH; n=60	7.47±2.76*&#	4.75±1.79*& #
Patients with CP and AH following treatment		
Patients with mild CP combined with AH; n=60	3.53 ±1.76	8.23 ±2.65
Patients with moderate CP combined with AH; n=60	4.03±2.45	7.26±3.19

Note: *the data is presented as M±SD; * — differences compared to the values in basically healthy individuals are statistically significant (p<0.05); & — differences compared to the values in patients with CP with no AH are statistically significant (p<0.05); # — differences compared to the values in patients with mild CP are statistically significant (p<0.05).*

combined, they reveal a mutually aggravating effect, where the endothelium is involved in the pathological process affecting the gum with changes in the gum neurotransmitter balance on the one hand, and a more significant endothelial dysfunction along with the combined effect of hemodynamic factors and a systemic inflammatory response — on the other. A success outcome here will take comprehensive dental treatment in combination with effective antihypertensive therapy.

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OSTEOPATHIC CORRECTION IN TREATING PATIENTS WITH TENSION HEADACHE SYMPTOM AGAINST TMJ DYSFUNCTION

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ABSTRACT — Patients may have headache due to pathological issues occurring in the cervical spine and resulting in compression of the vertebral artery passing nearby. This comes along with disturbed blood supply to the brain. The aim of this study is to offer reliable grounds, and evaluate osteopathic correction when treating headache of tension (HAT) caused by a dysfunction of the temporomandibular joint (TMJ). A comprehensive examination involved 26 patients aged 18 to 65. The study outcomes allow recommending to include soft osteopathic techniques in the set of therapeutic measures. In order to make osteopathic treatment available for patients with HAT caused by the TMJ dysfunction, computed tomography (CT) of the TMJ and cranio-cervical junction is a typical choice. Given the high efficiency of the combined work of an osteopath joining effort with an orthopedic dentist and an orthodontist who has a good command of neuromuscular correction approaches, it appears a feasible option to use such combined treatment in patients with the TMJ dysfunction-caused HAT. To improve the quality of osteopathic correction and maintain the positive outcome we recommend using a supporting teeth protector — an elastopositioning corrector or TMJ trainer. This enables to decompress the TMJ.

KEYWORDS — headache of tension, TMJ dysfunction, craniosacral system, osteopathic treatment.

INTRODUCTION

Headache may occur due to pathological processes affecting the cervical spine leading to a compressed adjacent vertebral artery, which is accompanied by a disturbance in the brain blood supply. Compressed nerves result in a violation of the neuromuscular impulse transmission. In view of this, the measures taken by an osteopathic doctor are to be aimed at eliminating the major cause of pain thus trying to prevent the development of a tension headache, and subsequently — a brain stroke. Osteopathy employs the method of relaxing tense tissues, while restoring the activity of all the structures that are clamped and compressed. Unlike manual therapy, osteopathy works not only with musculoskeletal diseases yet also corrects clamps in deeper systems of the body. A few painless procedures usually prove enough to eliminate the headache issue [10]. Headaches of tension (HAT) are the most common types of headache, which in most cases are a symptom of the TMJ dysfunction [2, 5, 8, 14]. The factors causing the TMJ dysfunction include head injuries and jaw tension through stress, gum chewing and biting a pencil/pen, which, in turn, results in muscle spasms, circulatory disorders, and all this combined can cause headaches [1, 4, 7, 12].

Inflammation of the joint and the surrounding muscles leads to the temporomandibular joint displacement, thus causing tension in the main masticatory muscles (pterygoid lateral, pterygoid medial, masticatory and temporal). As a result, there appears pain in the head caused by muscle tension [3, 9, 13, 15]. Treatment of tension headaches in orthodox medicine is mainly seen from the standpoint of medication treatment, which proves ineffective in case HAT is caused by the TMJ dysfunction — a powerful factor of the trigeminal nerve irritation. The term "HAT" reflects the theory claiming that it is the result of long tension of the head and neck muscles [6, 11]. However, not all patients suffering from this pathology feature high tension of the pericranial muscles.

In this regard, osteopathic correction when treating patients suffering from the tension headache symptom with a disturbed dysfunction of the temporomandibular joint and cranial system is a relevant measure since it will increase the treatment effectiveness and improve the patient's life quality.

Aim of the study:
to offer reliable grounds and evaluate osteopathic correction when treating cases of tension headache caused by the TMJ dysfunction.

MATERIALS AND METHODS

The comprehensive examination, which was carried out, involved 26 patients aged 18 to 65, among them 18 women and 8 men (Table 1), who had the symptom of tension headache caused by the TMJ dysfunction. As the table shows, females predominate in the pool of patients. This pathology was to be observed in fewer patients aged 18–30 if compared to those within the group of 31 to 65.

Table 1. *Distribution of the examined patients – age and gender*

Number of patients	Age, yrs		Gender	
	18–30	31–65	F	M/M
26 (100%)	7 (26.9%)	19 (73.1%)	18 (69.2%)	8 (30.8%)

The examination included collecting anamnesis of the disease; assessing the intensity based on the visual analog scale (VAS); postural examination and osteopathic testing of the musculoskeletal, craniosacral and visceral systems; neurological examination; consultation & examination of an orthopedic dentist; CT of the TMJ and of the cranio-cervical junction.

The duration of the disease (verified tension headache symptom) ranged from 3 months to 20 years. Around 45% of the patients had tension headache with weekly attacks. All the patients had periodical HAT since they had the temporomandibular joint dysfunction, as verified through CT and confirmed with dental and osteopathic examinations.

Based on the random sampling method, the patients were divided into two groups: the main group – 17 patients who underwent osteopathic correction at the stages of comprehensive treatment: dental, medical and physiotherapy, and the control group, which included 9 patients who underwent alternative treatment with no osteopathic intervention. The patients of the main and control groups had their disease anamnesis analyzed along with the specific features of the clinical manifestation, as well as they had CT scanning of the TMJ and the cervical-brain junction examined, with clinical and osteopathic changes identified and evaluated prior to, and following the treatment.

The diagnostics of patients with the tension headache symptom relied on one of the major examination methods — a thorough collection of the patient's complaints concerning the time of seeking help from an osteopathic doctor. In case of specific complaints registered, which were related to the localization of the maxillary system and the facial area, the TMJ dysfunction was assumed to be present, which allowed the osteopathic doctor to follow the examination protocol. Complaints presented by patients with the TMJ dysfunction are conditionally broken into two groups: a specific symptom complex that characterizes functional and morphological changes in the TMJ, and a non-specific symptom complex that describes the clinical picture, where symptoms related directly to the TMJ develop, yet going beyond the masticatory system — headache (forehead, occiput, temporal area, radiating pain), noise, ringing in the ears, vertigo, etc.

In order to assess the headache intensity, a ten-point visual-analog scale (VAS) was employed, where the patient registers the intensity of headaches within a certain period of the study.

The postural study is aimed at investigating the redistribution of the muscle tone and tonic responses that serve to ensure dynamic stabilization of the body in an upright position and detect the point where there was some imbalance of a complex mechanism.

Musculoskeletal dysfunctions affect the posture and the redistribution of the muscle tone in charge of the body vertical position. After the postural examination, a posturological diagnosis was set, i.e., the factor behind the pain syndrome development identified.

The evaluation of the craniosacral mechanism was carried out subject to a conventionally accepted osteopathic methods by examining and palpating the anatomical structures of the craniosacral system, namely, the skull and the sacrum. The skull was palpated under the arch based on the Sutherland method. The neurological examination was carried out according to the classical methods.

The dental examination was performed by an orthopedic dentist or an orthodontist aiming to diagnose the TMJ dysfunction. At the initial visit, the patient, jointly with the dentist, filled out the questionnaire form *Musculoskeletal and occlusive symptoms and signs*.

The data on the TMJ dysfunction was confirmed through computed tomography and X-ray examination. A diagnostic Galileos Sirona 3D cone-beam computed tomograph was used to perform computed tomography of the temporomandibular joint and the craniocervical junction. A 3D image allows obtaining any cross-section of the required area as well as any projection. Computed tomography was performed in patients of the main and control groups. The factors that were taken into account included: the position of the mandibular head in the articular cavity; the size and the asymmetry of the TMJ articular cavities; the mandibular head posterior displacement (disc compression); the symmetry of the atlanto-occipital joints articular cavities; the dens axis position.

The patients of the main group were given conservative treatment, which included muscle relaxants,

non-steroidal anti-inflammatory drugs, B vitamins, physiotherapy, general relaxing massage, physical therapy, dental correction. The treatment was administered and supervised by a neurologist, once a week on an outpatient basis.

The examination results were documented in the dental patient's personal medical record (each patient's functional norm, probability of dysfunction (risk group), and presence of dysfunction were registered).

RESULTS OF EXAMINATION

An analysis of the results obtained through registering the disease details revealed that the most common complaints presented were headache, pain in the cervical spine, ringing and tinnitus, sleep disorders, nervousness, vertigo, sounds in the TMJ when opening the mouth, pain in the face area, numbness in the fingers, restricted mouth opening (Fig. 1).

Following the VAS-based test outcomes, which will be obtained through the entire observation period for both main and control groups, the osteopathic doctor will analyze the course of pain attacks in general and evaluate the dynamics of treatment.

Fig. 2 shows the results of an osteopathic examination of the craniosacral system, TMJ, masticatory, supra- and sub-lingual muscles and ligaments.

Osteopathic observation revealed the most common issues in patients with HAT due to TMJ dysfunction: temporal muscle dysfunction (100% for both groups); hyoid bone dysfunction (88.2% for the main and 88.9% for the control group); decrease in the sacral force of the primary respiratory mechanism (PRM) (88.2% in the main group and 88.9% in the control group); masticatory muscle dysfunction (82.4% for the main and 88.9% for the control group); decrease in the cranial force of the PRM (82.4% in the main and 77.8% in the control group); dysfunction of the stylomandibular ligament (70.6% in the main and 77.8% in the control group); decrease in the PRM sacral amplitude (70.6% and 77.8%); reduced cranial amplitude of the PRM (70.6% and 66.7%).

The following issues were identified through a neurological observation (Fig. 3).

A dental examination by an orthopedic dentist revealed a correlation between the TMJ dysfunction and a change in the conventional occlusion (extraction of one or more teeth, depulpation, elevated fillings, poorly installed prosthetics, braces). CT studies showed certain changes in the joint topography and asymmetry of the masticatory muscles; besides, at the articular walls are displacement, microtrauma of the joint soft tissues occurred, as well as compression of tissues in certain areas and their stretching in other parts of the joint (compression and distraction). Computer tomograms do not distort the image of tissue structures, while both bone and soft tissues are visualized, i.e., the articular disk and masticatory muscles.

RESULTS OF TREATMENT

In the main group, the patients received conservative treatment, which was carried out in view of the diagnosed osteopathic disorders. Osteopathic rehabilitation in the main group was done once a week. The selection of the methods employed for each session depended on the osteopathic disorders and on the dynamics of the disease clinical manifestations. The number of sessions depended on the neurological and osteopathic status, and varied from 5 to 8 sessions. An average rehabilitation period was 1.5–2 months.

The sequence of the treatment offered to the patients in the main group was as follows: first, the TMJ trainer was administered (supporting teeth protector to decompress the TMJ) with osteopathic treatment performed at the same time; further, the patients were referred to the dentist to fix the result through an orthotic made individually in view of the neuromuscular occlusion. In some cases, after wearing an orthotic, patients were given prosthesis or had a repeated prosthesis, still taking into account their respective neuromuscular occlusion.

Based on the osteopathic concepts, aiming to complete the tasks set herein, we found it important to release all the anatomical structures underlying the muscle, fascial, bone and visceral dysfunctions in the body that can affect the mandibular joint function. Osteopathic treatment started with the release of the sacrum in relation to the L5 vertebra and the release of the cranio-vertebral junction, followed by the release of the thoracic diaphragm, the release of the upper aperture, the correction of the jugular foramen, the large occipital foramen, the membranous equalization of the skull and, in some cases, the venous sinuses drainage.

Based on the data obtained from the anamnesis, the neurological and osteopathic examination, as well as CT data, respective treatment was caried out, which included several major stages:

Stage 1. A global approach to treating HAT, which appeared as shown below:

1. Recovered sacral mobility in the sacroiliac joints, L5–S1.

2. Elimination of dysfunctions in the thoracic, pelvic diaphragms and upper thoracic aperture.

3. Elimination of cervical spine dysfunctions, specifically levels C0–C1. Balancing the neck deep fascia, as well as restoring the free kinetics of the clavicles and the first ribs.

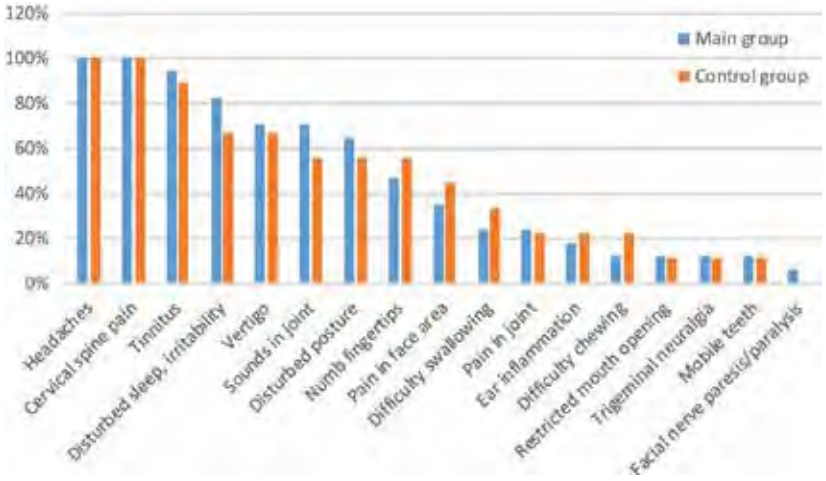

Fig. 1. *Complaints reported by patients in main and control groups, %*

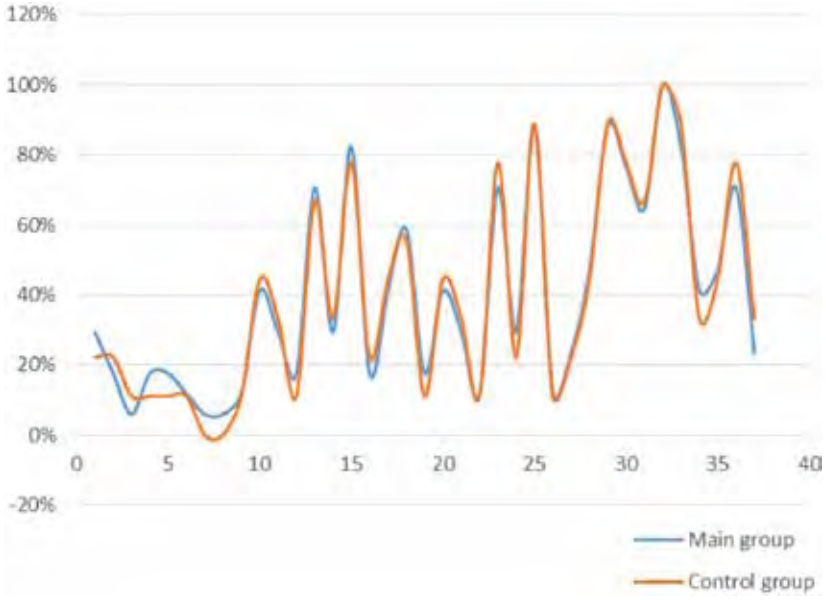

Fig. 2. *Results of an osteopathic observation prior to treatment, main and control groups*

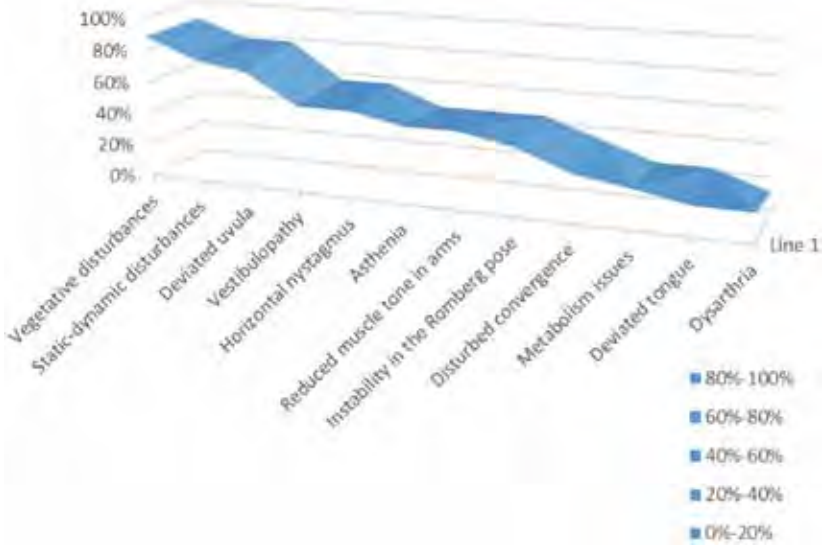

Fig. 3. *Neurological status of patients with HAT at their first visit to medical experts*

4. Elimination of sphenobasilar synchondrosis dysfunction, correction of the skull base sutures (occipito-mastoid suture, petro-jugular, petro-basilar).

5. Inhibition of the upper and lower cervical sympathetic ganglia.

6. Drainage of venous sinuses.

Stage 2. Correction specific techniques:

— masticatory muscles treatment;
— hyoid muscles treatment and the hyoid bone correction;
— lower jaw ligaments treatment (sphenoid-mandibular ligament, stylomandibular ligament);
— treatment of petrosphenoid ligament and sphenopetrosal synchondrosis;
— nasal breathing improvement: treatment of paranasal sinuses and tonsils.

CT results prior to, and following the treatment in the main and control groups

The studies were carried out using a Galileos Sirona diagnostic 3D cone-beam computed tomographic scanner. If compared with the previous tomogram image, there is a positive dynamics to be seen in recovering the symmetry of the temporomandibular joints articular cavities.

If matched against the previous tomogram of the craniocervical transition, the symmetry of the C0–C1 and C1–C2 articular cavities appears restored, while the asymmetric position of the dens axis — relative to the atlas lateral masses — is eliminated.

As far as our work is concerned, the most notable was the change in the articular cavity size after the treatment, as well as the improved position symmetry of the dens axis as well as the position between C0–C1, C1–C2, both on the left and on the right.

As can be seen from the table above, positive changes in the cranio-cervical junction joints are more pronounced in the main group. The change in C0–C1 was 66.67% (38.89% for the control group), C1–C2 — 74.27% (30.83% for the control group), the distance difference from the dens axis to the atlas lateral masses decreased in the main group by 50.48% (42.49% for the control group).

As the CT data shows, uni- or bilateral TMJ compression was identified in the main and control groups of patients with HAT caused by the TMJ dysfunction.

In most cases, patients with the TMJ dysfunction featured an asymmetry of the temporomandibular joint articular cavities. In the Galileos software program, the radiologist measured the articular cavities on the left and right before and after the treatment. The obtained data served to calculate the asymmetry value before and after the treatment. Due to the treatment, the asymmetry decreased, whereas the change was statistically significant (Table 3).

Since an approximately equal number of patients featured the left and right joints compression (47% and 53%) among those with asymmetry in the TMJ articular cavity (both in the main and in the control group), the calculation of the average articular cavity values of the left and right TMJ would prove not reliable in view of the aims set for this study. We calculated the average articular cavity before and after the treatment to find out that the average value after the treatment in the main group was 4.45 mm, which is 47.8% above the initial value of the articular cavity. As far as the control group is concerned, the result proved similar — the change made up 44.9% (Table 4). These results point at joint decompression after the treatment in both groups.

Table 2. *Average values of the cranio-cervical transition asymmetry, prior to and after the treatment*

Parameter	Group	Prior to treatment, mm (M±m)	Following treatment, mm (M±m)	Change, M	Reliability of difference
Δ of C0-C1 distances	Main group (n=17)	0.51±0.26	0.17±0.11	66.67%	p<0.05
	Control group (n=9)	0.54±0.29	0.33±0.22	38.89%	p>0.05
Δ of distances from dens axis to the atlas lateral masses	Main group (n=17)	2.08±0.77	1.03±0.15	50.48%	p<0.05
	Control group (n=9)	1.93±0.55	1.11±0.21	42.49%	p<0.05
Δ of C1-C2 distances	Main group (n=17)	1.71±0.84	0.44±0.32	74.27%	p<0.05
	Control group (n=9)	1.2±0.52	0.83±0.21	30.83%	p>0.05

The dynamics observed on the computed tomography images before and after treatment in the main and control groups can be seen from Table 2.

The following changes could be observed in both the main and in the control groups following the treatment:

Table 3. *Average asymmetry in the articular cavities of the left and right TMJ, prior to and after the treatment*

	Prior to treatment	Following treatment	Reliability of difference
Main group (n=17)	0.6±0.23	0.47±0.25	p<0.05
Control group (n=9)	0.58±0.24	0.46±0.26	p<0.05

Table 4. *Average values of the TMJ articular cavities, prior to and after the treatment*

	Prior to treatment, mm	Following treatment, mm	Change, %	Reliability of difference
Main group (n=17)	3.01±0.88	4.45±1.03	47.84%	p<0.001
Control group (n=9)	2.94±0.89	4.26±1.04	44.90%	p<0.001

—reduced asymmetry in the TMJ articular cavity as well as an increase in its size on both sides (joint decompression);

—aligned dens axis tooth relative to the atlas lateral masses;

— aligned C0–C1 and C1–C2 distances.

These changes are statistically significant for the main group. As for the control group, they manifested there to a lower extent. An increase in the articular cavity size as well as a decrease in its asymmetry from left to right as a result of treatment entailed a distinctly improved status of the cranio-cervical junction (aligned dens axis relative to the atlas lateral masses, a better symmetry of the C0–C1 and C1–C2 articular cavities).

To be noted also that the correlation between TMJ dysfunction and the joint dysfunction of the cranio-cervical junction was observed in 72% of the cases in the two groups.

The dens axis deviation was found in all patients with significant asymmetry of the TMJ articular cavities. There was no clear correlation between the TMJ compression and the dens axis deviation in either direction. This, as we see it, is due to the body's different potential to adjusting to such structural issues.

Changing HAT intensity before and after the treatment was statistically significant for both groups, whereas the difference between the average tension headache intensity after the treatment in the groups was statistically significant as well, with a probability degree of p<0.001. (Table 5, 6).

It should be noted that in the absolute majority of cases, the patients did not get rid of headaches — the pain intensity went down, as well as the frequency and the duration of seizures decreased. The average score of the HAT intensity, estimated on the visual analog scale in the main group dropped from 8.3 to 4.9 (by 41%). The frequency of HAT attacks in the main group decreased from 1 time per week to 1 time per month. The duration of seizures in the main group prior to the treatment was 24 hours and 8 hours — after the treatment.

The patients in the control group revealed lower results. The decrease in the average HAT intensity (VAS scale) was 31%. The seizures frequency decreased from 1 time a week to 2 times a month. The seizures duration changed from 24 hours to 15 hours for the entire group on average.

CONCLUSION

The 26 patients with tension headache had a dysfunction of the temporomandibular joint, which was verified by CT and confirmed by dental and osteopathic examinations, as well as the said patients suffered from periodic HAT attacks. A comparative assessment of the patients' status dynamics was carried out 1 month following the completion of the treatment course. The assessment focused on complaints (either reported or lacking), CT data of the TMJ and the cervical-brain junction, neurological and osteopathic statuses. The osteopathic correction of HAT caused by the TMJ dysfunction reveled a significantly improved clinical presentation, subject to the neurological indicators dynamics. The achieved improvement was most significant in the main group for the following syndromes: asthenia — from 47% to 6% (no dynamics observed in the control group); vegetative disorders — from 88% to 19% (in the control group – from 89% to 44%); dysarthria — from 18% to 0% (no dynamics observed in the control group); horizontal nystagmus — from 53% to 0% (in the control group — from 56% to 11%); vestibulopathy — from 53% to 13% (in the control group — from 56% to 22%).

In view of the above, the osteopathic correction produced more significant outcomes for the dynamics of neurological indicators. The HAT intensity before and after the treatment was assessed by VAS, where the difference between the average tension headache intensity after the treatment in both groups was statistically significant, too.

It should be noted that in the absolute majority of cases, headaches never disappeared completely – the pain intensity went down, as well as the frequency and the duration of seizures decreased. The HAT intensity average score, estimated on the visual analog scale dropped from 8.3 to 4.9 (by 41%) in the main group. The HAT attacks frequency in the main group went

Table 5. HAT average values, prior to and after the treatment, M±m

	Prior to treatment	Following treatment	Reliability of difference
Main group	8.3±0.8	4.9±1.1	p<0.001
Control group	8.4±0.9	5.8±1.4	p<0.001

Table 6. HAT intensity average values in the main and in the control groups following the treatment, M±m

	Main group (n=17)	Control group (n=9)	Reliability of difference
HAT intensity, VAS scale (points)	4.9±1.1	5.8±1.4	p<0.001

down from 1 time per week to 1 time per month. The duration of seizures in the main group prior to the treatment was 24 hours and 8 hours — after the treatment.

The results in the control group were not so significant — the decrease in the average HAT intensity (VAS) was 31%. The seizures frequency decreased from 1 time a week to 2 times a month. The seizures duration changed from 24 hours to 15 hours for the entire group on average.

Given the above, patients with HAT caused by TMJ dysfunction need an osteopathic diagnostics procedure, for the craniosacral system above all. Based on the study results we can recommend including soft osteopathic techniques in the entire set of treatment measures.

To make the osteopathic treatment offered to patients with the TMJ-dysfunction-bound HAT, CT of the TMJ and cranio-cervical transition are recommended. Given the high efficiency produced by combining an osteopath's effort with an orthopedic dentist who has a command of the neuromuscular correction methods, it is advisable to act jointly when treating patients with HAT caused by the TMJ dysfunction. To improve the osteopathic correction outcomes and maintain the achieved results we recommended using a TMJ trainer — a TMJ supporting and decompressing teeth protection appliance.

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AIDS AND METHODS FOR PERSONAL ORAL HYGIENE IN PATIENTS WITH NON-REMOVABLE ORTHODONTIC APPLIANCES

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ABSTRACT — The demand for aesthetic dental rehabilitation, and respectively, for a higher quality of life, is growing each year. This entails a growth in the number of orthodontic corrections employing non-removable appliances. A high risk of developing periodontal issues while undergoing treatment reveals that orthodontist need to have all-round comprehensive knowledge of the tactics, the principles and the methods of hygiene measures. A high level of the doctor's awareness along with their ability to motivate the patient, would allow them to arrive at successful doctor-patient relationship not only when dealing with orthodontic diseases, yet also in terms of maintaining the periodontium health due to a thorough approach to the issues of preventive oral hygiene during orthodontic treatment.

KEYWORDS — orthodontics, periodontitis, oral hygiene, patient motivation.

INTRODUCTION

Orthodontic correction is gaining more and more of relevance and demand as a field of comprehensive dental rehabilitation [1–4]. The global technological progress and growing awareness entail an increase in the number of visits to dentists' offices not only to get help in treating a particular pathology, but also seeking some dental aesthetic correction thus to enjoy a higher quality of life [5–8]. A large number of research papers focusing on the relationship between individual organs and systems never fail to note the fact that the proper functioning of the dental system features a direct relation to systems like the gastrointestinal tract, cardiovascular system, respiratory system, etc. Improving a pathological occlusion and developing a proper interdental and inter-maxillary contacts can improve the patient's overall health and add to their psychological comfort [9–12].

The non-removable appliance technique, which — individually or in combination with other auxiliary elements — can help eliminate complex dental issues, still remains the most common method employed to treat adult orthodontic issues nowadays, the global prevalence of this method reaching 11.4–71.7% [13, 14]. One of the important criteria to ensure success of the respective treatment is the enamel and teeth periodontium status. Researchers have developed numerous methods and tools helping maintain the oral cavity health and prevent dental diseases [15].

Failure to comply with the doctor's recommendations during a serious long-term course of orthodontic treatment may lead to various transformations affecting the patient's oral cavity, namely, damaged periodontal tissues and tooth enamel [16]. Researchers claim that periodontal diseases are rather common observed in patients with non-removable orthodontic appliances, the said issues accounting for about 20–38% of all disorders, which reflects data offered by other authors, who, though, report a much higher occurrence rate — up to 92%, and yet there are even more data sources claiming even higher a rate — up to 99%. Speaking of patients with non-removable orthodontic appliances, the probability of caries makes up 15% to 85%. This high level of prevalence of the above-mentioned dental diseases combined with treatment approaches involving non-removable orthodontic appliances could be attributed to an unclear clinical presentation at the initial stages of the disease progress, which entails difficulties in diagnostics and, consequently, the lack of timely corrective intervention. Catarrhal and chronic hypertrophic gingivitis signs are to be observed as soon as 6 months into orthodontic treatment, whereas the entrance here is the dental grooves, accumulating a large number of microorganisms [17, 37].

The large volume taken by non-removable orthodontic elements placed for a long correction period on both the vestibular and the oral surfaces of the teeth, results in difficulty experienced by patients through daily oral cavity care procedures. Braces are most commonly located on the central part of the tooth crown, or they can be shifted towards the gum, while their design includes wings and hooks. These elements make daily hygiene even more difficult, since conventional

toothbrushes no longer allow cleaning plaque out of hard-to-reach spots. As a result, these spots turn to be the areas where plaque or biofilm accumulate in large amounts, with periodontal inflammation and demineralization taking a more intense course, getting localized around the bracket base, all this finally turning into a carious cavity [19, 31]. The oral microbiota composition undergoes certain change in the presence of abundant plaque along with a growth in the pathogenic microflora, which in turn will change the periodontium structure thus causing its inflammation [22]. Preventive hygiene measures are an important factor in an attempt to solve the issue successfully: these measures include teaching the patient the rules of routine oral and dental hygiene, individual selection of a toothbrush, a paste, a rinse aid, an irrigator, a floss as well as other hygiene products and techniques, and of course a high motivation to arrive jointly at the desired treatment outcome [18, 32].

Edema, gum hyperemia, itching and soreness when brushing the teeth, bad breath — all these are the first symptoms signifying the onset of changes affecting the periodontium in patients with non-removable orthodontic appliances. All the patients involved in the study carried out by A.V. Lopatina et al. were observed to have catarrhal gingivitis. The author also notes that the PMA index went 40% down if compared to the initial value [29]. The monograph by Soboleva T. Yu., contains the following recommendation: when positioning braces, bondage rings, it is advisable to act with extreme accuracy when filling the teeth fissures with a composite, leaving no space between them and the enamel. If done otherwise, there will be areas of orthodontic appliance loose fitting, which will accumulate plaque, all this eventually leading to the dental enamel demineralization. Also, the excessive fixing material should be removeв carefully, this done to protect the periodontium against chronic injury [35].

Given high-precision, accurate and careful work performed by the doctor, an important factor is involving the patient in the treatment process, which is of a continuous course. This serves a sufficient explanation to the need of maintaining due regular daily dental care. It is important to enhance people's awareness, to motivate patients undergoing orthodontic treatment encouraging them to use permanently various preventive and medical oral care products. The relevance of this issue is obvious, due to which a clear task is to be defined here for each orthodontist, namely, taking an individual approach not only from the stance of the purely orthodontic aspect when correcting the respective major pathology pertaining to the doctor's field of practice, yet also adopting more extensive clinical thinking in view of preventing the negative effects of the patient's negligence in matters of daily hygiene routine, as well as aiming to assume joint responsibility for the treatment outcomes [23, 26].

A proper systematic approach to hygienic and preventive procedures will reduce the risk of developing dental diseases. The abundance of individual oral hygiene means and methods available nowadays will often leave the patient confused, so the orthodontist's task is not only to select the right hygiene products for a particular patient, yet also to teach them how to use the products in question. The most popular means used in orthodontic practice when employing non-removable appliances include pastes with a therapeutic and preventive effect containing rich in fluoride and enzymes; toothbrushes with a special groove for braces; electric toothbrushes with a special orthodontic nozzle; end-tufted and interdental brushes; flosses, irrigators, tongue scrapers, rinses and elixirs [30].

71% of patients using special means can maintain personal oral hygiene at the proper level. When starting orthodontic treatment, the doctor teaches the patient the rules of brushing teeth (standard way). The authors' study reports that the hygiene index in patients through orthodontic treatment fluctuated from HI=1.0 to HI =1.5, whereas the PMA index fell within the range of 0.4 to 0.6. If compared other patients, then those using standard brushes to clean plaque out of the oral cavity had lower indicators, HI=2.0–2.5, PMA=1.0–2.0. In the authors' publication, the Parma PMA index was near zero, whereas 1 month into orthodontic treatment, it went 25.3% up, which points at mild gingivitis [28].

The main tool in terms of dental care are brushes that differ by the material their bristles are made from, the stiffness, the number of bundles, the texture, the shape of the bristles cut, etc. Besides, brushes come out as manual or electric. Authors writing about the effectiveness of toothbrushes in orthodontic patients mention an interesting fact. The author mentioned above claims that after using electric brushes for 2 months, the hygienic and gingival indices proved significantly below those ($p < 0.01$) observed in cases where manual brushes were used, which is in line with personal observations when using such self-administered gadgets with no due professional advice [21]. Ivanov V. Yu. et al., in turn, note in their article that the quality of tooth brushing does not depend on the type of the toothbrush used, while good manual skills in consistently removing plaque from all the teeth surface can ensure highly effective prevention of major dental diseases, which is also confirmed through daily practice observations [27].

Interdental thread (floss) is used to clean the interdental spaces. Fleischer G. M., in an article pub-

lished in 2019 and focusing on a specific narrow topic claims that flosses containing fluoride components contribute to a faster recovery of the dental sulcus pH after meals, bringing the said indicator's values in the dental fluid back to neutral. This fact can be well employed in clinical practice, since it is the interdental contacts that often become the starting point for the infection development, manifesting itself both as hidden carious cavities and as dental and gingival pockets, which in turn progress into extensive lesions over a long period of orthodontic treatment [24].

The selection of toothpastes for periodontal diseases and caries is to be done on a strictly individual basis [20, 38].

In the article [33], the group of authors analyzes the value of all hygiene products, as well as they analyze several ways to clean the oral cavity. The authors point at the S.B. Ulitovsky method as the most effective one, which includes several stages: preliminary irrigation of the oral cavity; cleaning the interdental spaces with a floss; repeated irrigation; cleaning the retromolar area and the teeth fissure with an end-tufted brush. Further on, the recommendation suggests using a paste and a brush with a V-shaped protrusion to clean all the dental surfaces, after which the mouth is to be rinsed again, followed with an interdental brush cleaning of the dental areas between the non-removable appliance. After that the oral cavity should be irrigated with a mouthwash. The safest products are based on natural components, so their use does not involve the risk of developing dysbiotic phenomena in the oral cavity as well as ensures a positive dynamics of the hygienic indicators along with a significant inflammation reduction in the periodontal tissues [25]. Further, it is recommended to clean the tongue with a scraper, after which an irrigation is to be carious out intermediately, then cleaning with an end-tufted brush around the bracket base, and a final irrigation of the oral cavity with a rinse for therapeutic and preventive purposes. Mouth rinses containing xylitol have proven to reduce plaque formation through impeding the activity of Streptococcus mutants in saliva, proof to that offered in Dr. Satygo's article [34]. Remineralizing therapy is recommended both during and following orthodontic treatment with a non-removable appliance [36, 39].

CONCLUSION

The presented reference data provides evidence to the relevance of studies focusing on comprehensive preventive measures through all stages of orthodontic treatment. Despite recent advances, oral hygiene still remains an issue of note. Poor oral hygiene leads to accumulation of dental deposits, whereas non-removable orthodontic appliances may trigger inflammation in the periodontal tissues and the mucous membrane. This result in lower local immunity, increased pathogenetic activity of the microflora, shifts in the oral homeostasis and to caries-related disorders.

The onset and progression of dental diseases in orthodontic patients requires further in-depth study of this problem with a focus on improvement of overall dental health, and enhancing preventive as well as treatment strategies.

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MORPHOMETRIC PATTERNS OF MAXILLARY APICAL BASE VARIABILITY IN PEOPLE WITH VARIOUS DENTAL ARCHES AT PHYSIOLOGICAL OCCLUSION

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ABSTRACT — Morphometric data on the structure of the craniofacial complex are reliable and diagnostically significant values that are of applied nature in terms of practical dentistry. Within this study, analysis of cone-beam computed tomograms, biometric indicators of plaster models obtained from the jaws of 83 people (aged 21–35) with physiological occlusion and various types of dental, gnathic dental arches, the degree of proportion between the maxillary apical base and the inter-canine distance were identified. Depending on the dental arch type, the patients were divided into three groups. The morphometric study in the CBCT frontal plane was the distance between the canines tearing tubercles and the inter-canine distance in the apical area. The study outcomes revealed discrepancies between the calculated and actual indicators of the apical base width for all types of dental arches in people with physiological occlusion. In case of mesotrusive dental arches (incisional angle — 127–143°), the width of the apical base corresponded to the width of the dental arches between the canines, while the differences in indicators were not statistically significant. In people with retrusive dental arches (incisional angle exceeding 144°), the width of the dental arch was found to be significantly above the width of the apical bases. As far as protrusive dental arches are concerned (incisional angle below 126°), these patients featured predominance of the apical bases width over the inter-canine distance. The obtained data add to that already available in research literature regarding the relationships and dimensional features pertaining to the craniofacial complex structures, as well as have applied value in orthodontic clinical practice.

KEYWORDS — morphometry, maxillary apical base, cone-beam computed tomography, inter-canine width of the dental arch, physiological occlusion, dental arch, craniofacial complex.

INTRODUCTION

Studying the craniofacial structures should be performed from the individual and age-related variability stance. This area allows, in each specific case, identifying the features of the structure and the position of a particular anatomical area in the craniofacial complex relying on a set of signs [3, 8, 14, 18, 21, 23, 36, 44]. Given that, studying the relationship between dental arches and craniofacial parameters is an urgent issue, which is associated with employing advanced orthodontic technology in treating patients with various dentition and occlusion issues [2, 9, 16, 19, 25, 32, 41, 47].

The attention focus, for both anatomists and clinicians, is dental arches. On the one hand, they are involved in the development of the facial area of the skull, while on the other — they are the object of manipulation for orthodontic and orthopedic dentists. The data obtained through investigating the variant anatomy of dental arches serve the basis for comprehending the patterns involved in the structural arrangement of the facial area of the skull as a whole [10, 12, 20, 22, 26, 35, 54].

The apical base of the jaws is an anatomical structure, defined as a conditional line connecting the tops of the teeth roots. In clinical orthodontic practice, measurements of the length and the width of the apical base are an inevitable part of diagnosing maxillofacial anomalies and deformities [1, 4, 7, 24, 48]. The main methods used for assessing the parameters of the apical base mentioned by Howes and N. G. Snagina can be seen from respective manuals and textbooks on orthodontics and are based on measuring the width of the 12 teeth crowns [5, 11, 27].

The apical base width has been proven to be determined by the teeth inclination in the vestibular-lingual direction, which in orthodontics is called the teeth torque. In view of this, quite logical is to conclude that at high torque values, the tooth crown is inclined more towards the vestibular side, while the root is more inclined towards the lingual side, if compared to cases of low inclination angle values. The torque values for various types of physiological occlusion are available in respective works published by most specialists [6, 17, 42, 45, 49].

As the authors claim, the parameters of the apical base width depend on the anatomical facial features. There has been a direct dependence shown between the width of the maxillary apical base and the morphological width of the face. Notable is that this work offers data in view of the face belonging to the wide, medium or narrow types, with no regard as to the gnathic features of the maxillofacial area [31].

Literature offers information showing that people with the brachygnathic type, have a wider shape of the upper dental arches in the transversal, and shorter – in the sagittal plane, if compared to mesognathic dental arches [30]. Research involving people with dolichognathic types showed a whole opposite situation, namely, the upper dental arches were shorter in the transversal plane and longer in the sagittal, if matched against mesognathic dental arches [34, 37]. However, in the studies mentioned above the authors offer no details on the parameters of the apical base in persons with different types of face and dental arches.

There are some advanced approaches to studying dental and alveolar arches, including cone-beam computed tomography, presented [13, 28, 39, 50]. These approaches are recommended for their use in clinical dentistry to diagnose respective pathologies and guide the treatment choice [33, 38, 43]. During that, there is no data on the size of the apical bases and their relationship with the width of the dental arch between the canines, which explains the aim of this study.

Aim of study:
to identify the proportion of the maxillary apical base to the inter-canine distance in people featuring various types of dental arches with physiological occlusion.

MATERIALS AND METHODS

A stratified retrospective study was carried out, where cast models and cone-beam computer tomograms (CBCT) were studied as obtained from 83 patients within their first mature age (21–35 y.o.) with physiological occlusion and various gnathic dental types of arches. The entire body of patients was broken into groups in view of the dental arches erosive type. 29 of the patients had mesotrusion, another 26 — protrusion, with 28 more featuring a retrusive type of dental arches.

The type of dental arch was identified subject to the incisional angle on tomograms. An incisional angle ranging between 127 and 143° corresponded to the mesotrusive type of dental arches. An increase in the incisional angle making it finally exceed 144°, allowed attributing the respective dental arches to retrusion, whereas a decrease in the angle reducing it to below 126° allowed including the dental arch to the protrusive type (Fig. 1).

The proposed criteria are in line with the data mentioned by the majority of experts studying the features of dental arches in case of physiological occlusion [29, 46, 51, 52, 55].

The levels of the canines and apical bases were identified through the CBCT in the transversal plane in the projection of the roots between the canines and the first premolars. Further, the distance between the canines cutting tubercles in the apical area was measured on the CBCT in the frontal plane (Fig. 2).

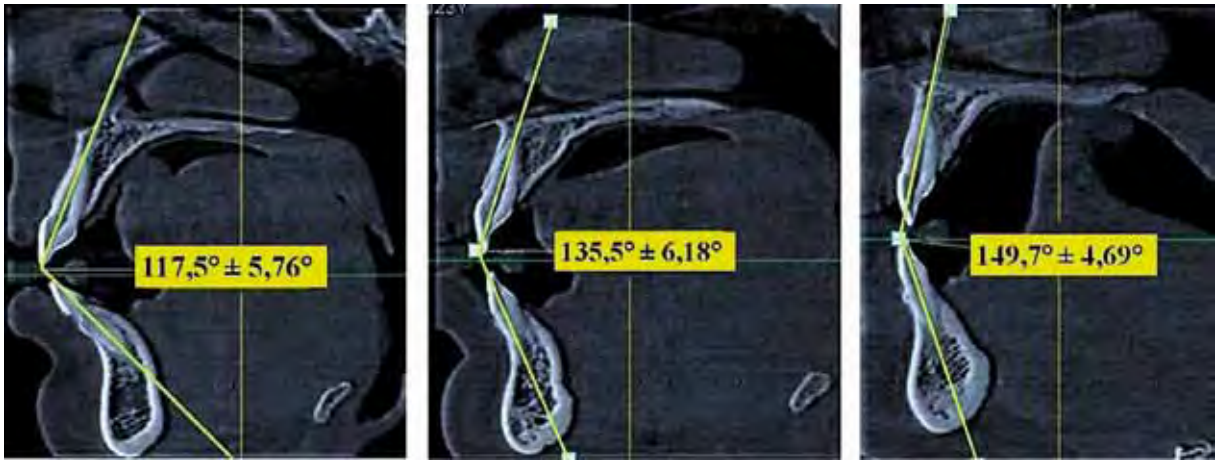
The study implied investigating the biometric indicators of jaw cast models. The odontometric measurements focused on evaluating the total width of the crowns of 14 and 12 teeth that make up the dentition. The sizes of 6 front teeth were measured. The odontometric data was used to calculate the types of dental arches, thus to determine whether the dental system belongs to the macro-, micro- or normodental type. Besides, the total size of the 12 upper teeth allowed calculating the apical base width, which normally (Snagina N. G., 1965) made up 44% of the obtained value. In the upper jaw models, the apical base was detected in the root tips projection between the canine and the first premolar. The actual value was compared with the calculated values for all types of dental arches.

The width of the dental arches between the canines was measured, where the points on the tearing tubercles were the landmarks. The dental arch width between the second molars was measured between the distal tubercles of the antimeres.

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

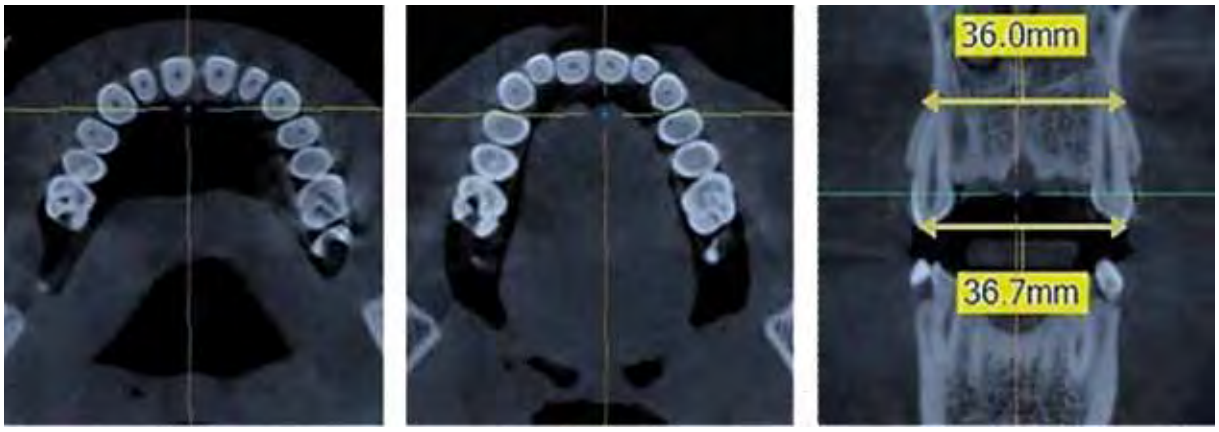
RESULTS AND DISCUSSION

A morphometric analysis of cone-beam computed tomograms and biometric indicators of the jaw cast models revealed that in case of physiological occlusion, the length of the dental arch, calculated as the sum of the width of the 14 teeth crowns, featured statistically significant differences depending on the type of arches. People with the protrusive type of dental arches, for instance, had the parameter in question significantly exceeding that in people with retrusion. This can be accounted for by the fact that in case of the protrusive arches, macrodontic dental arches were more common, while with the retrusive type the common type of dental arches was that of microdontia. Given that, the odontometric parameters of the 12 teeth and the 6 front teeth of the upper jaw featured certain differences, which can be seen from Table 1.



a b c

Fig. 1. *Images of the CBCT fragments with printed results of the incisional angle in case of dental arch protrusion (a), mesotrusion (b) and retrusion (c)*



a b c

Fig. 2. *The level of the CBCT sections aimed at identifying the canine distance (a) and the apical base (b) of the upper jaw, as well as the method for measuring the respective parameters (c)*

Table 1. *Transversal dimensions of the dental arches and apical bases (AP) of the upper jaw in people with physiological occlusion (M±m), (mm), ($p \leq 0.05$)*

Upper arch parameters	Size parameters at various arch types:		
	mesotrusion	protrusion	retrusion
Sum of 14 teeth	112.88±2.54	119.88±2.21	109.82±2.37
Sum of 12 teeth	93.70±2.06	99.31±1.98	90.89±2.13
Sum of 6 front teeth	46.63±1.18	48.68±1.32	44.93±1.09
AP calculated width	41.23±0.21	43.70±0.29	39.99±0.27
AP actual width	37.69±0.24	35.41±0.27	38.14±0.22
Width between the canines	37.22±0.27	35.96±0.28	36.72±0.25
Width between molars	60.62±1.49	63.02±1.54	63.30±1.62

In view of the differences in the total size of the 12 teeth, the differences in the apical bases calculated width in people with different types of dental arches were identified.

In people with the mesotrusive type of dental arches, where the total width of the 12 teeth crowns is 93.70±2.06 mm, the width of the apical bases, according to N. G. Snagina, should make up an average of 41.23±0.21. During that, the actual value measured on the models was 37.69±0.24 mm. The difference between the calculated and the actual values of the apical base width was 3.54±0.12 mm.

In people with protrusive dental arches, with the sum of the crowns width of the 12 teeth is 99.31±1.98 mm, the estimated width of the apical bases was 43.70±0.29 mm on average. The actual value, though, measured on the models, was 35.41±0.27 mm. The said difference between the calculated and the actual value was 8.3±0.17 mm, which exceeded significantly that in people with the mesotrusive type of dental arches ($p \leq 0.05$).

As for people with the retrusive type of dental arches, where the sum of the crowns width of the 12 teeth was 90.89±2.13 mm, the apical bases estimated width was 39.99±0.27 mm. At the same time, the actual value measured on the cast models was 38.14±0.22 mm. This *the calculated vs. the actual value* difference in the apical base width was significantly smaller ($p \leq 0.05$) than in the other study groups, making up 1.85±0.41 mm.

Special attention has been paid to the differences between the width of the apical base and the width of the dental arch in between the canines. The difference in size has been shown to depend on the type of dental arches (Fig. 3).

the difference in size being 0.47±0.19 mm. We have detected no significant difference between the width of the apical base and the inter-canine distance ($p \geq 0.05$).

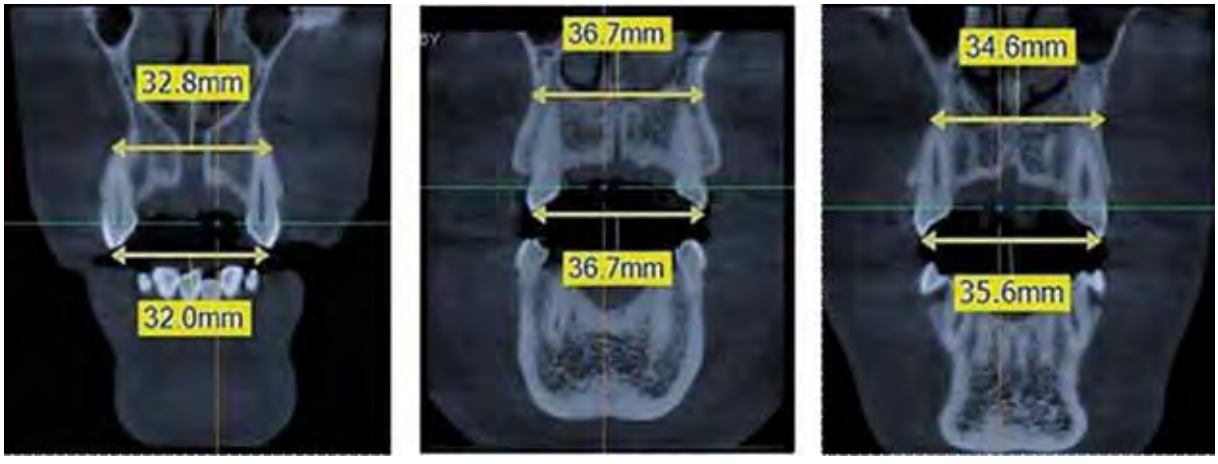
The actual value of the apical bases in the retrusion types of arches exceeded the value of the inter-canine distance (36.72±0.25 mm), whereas the size difference was 1.43±0.31 mm.

The actual value of the apical bases in case of the protrusive types of dental arches, in contrast to patients with the other types of arches, was smaller than the width of the dental arch between the teeth (35.96±0.28 mm), with the difference in size being 0.55±0.13 mm.

The outcomes of the study, therefore, revealed that there was a mismatch between the calculated and the actual indicators of the apical base width for all types of dental arches in people with physiological occlusion. The largest mismatch value, namely, 8.3±0.17 mm, was to be observed in those featuring the protrusive type of dental arches, the distinctive for them being *high* values of the canine torque, which is consistent with the opinion expressed by respective specialists [15, 40, 53, 56].

The differences between the calculated and the actual parameters of the apical base width were the smallest in people with the retrusive type of dental arches, the average difference making up 1.85±0.41 mm.

During that, the transversal dimensions of the maxillary apical bases proved to be close to the dimensions of the dental arches between the canines, yet



a *b* *c*

Fig. 3. *The relationship between the apical base size and the inter-canine distance in people with dental arches belonging to the retrusive (a), mesotrusive (b) and protrusive (b) types*

The actual value of the apical bases in case of mesotrusive dental arches (37.69±0.24 mm) was close to the value of the inter-canine distance (37.22±0.27 mm)

there were certain differences in the morphometric parameters of the studied groups.

CONCLUSION

1. The data obtained through studying biometric indicators of jaw cast models and cone-beam computed tomograms of patients with a full set of permanent teeth, physiological occlusion and various gnathic dental types of arches, point at a proportion between the morphometric parameters of the maxillary apical base width and the inter-canine distance.

2. In people with the mesotrusive type of dental arches, the actual width of the apical base (37.69±0.24 mm) matches the width of the dental arches between the canines (37.22±0.27 mm), whereas the difference in indicators does not come up to statistically significant values ($p \geq 0.05$).

3. Patients with the retrusive type of dental arches have the apical base actual width (38.14±0.22 mm) exceeding statistically ($p \leq 0.05$) the width of the dental arches between the canines (36.72±0.25 mm), while the difference in size is 1.42±0.31 mm.

4. In people with the protrusive type of dental arches, the apical base actual width (35.41±0.27 mm) falls slightly below the value of the width of the dental arches between the canines (35.96±0.28 mm), the size difference being 0.55±0.13 mm.

5. Further advance of algorithms employed to study the variant anatomy of dental arches, the apical bases of the jaws in the cranial structure, in view of the patient's individual features, would allow standardizing the methods of dental research, as well as modifying the generally accepted analysis and interpretation systems of the data obtained for reliable identification of patients with abnormal and deformed dental system.

6. The calculated data on the proportion of the maxillary apical basis and the inter-canine distance in people featuring different dental arches and physiological occlusion allow systematizing craniometric and odontometric study outcomes, obtaining reliable significant information on the patterns of the dental arch structure and their compliance with the morphometric specifics of the maxillofacial area, whereas such data is of value for research and clinical practice.

7. The inclusion of information on the existing (lacking) mismatch between the apical base width and the inter-canine distance in people with physiological occlusion and various types of dental arches into the *Clinical protocols for the diagnosing and orthodontic treatment of dental anomalies in outpatient setting* will help reduce the time spent by orthodontists through various stages of clinical examination and diagnostics, increase the effectiveness of the diagnosing dental issues, optimize the planning of orthodontic treatment, in particular, determine the location of the permanent canines in a position matching the apical base size.

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GUM FLUID BIOMARKERS IN PERSONALIZED DIAGNOSTICS OF INFLAMMATORY PERIODONTAL DISEASES

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ABSTRACT — Aim of the study: To evaluate the diagnostic value of crevicular fluid immunoregulatory biomarkers in cases with inflammatory periodontal diseases. The study included a total of 97 patients aged 21 to 55 years with inflammatory periodontal disease (gingivitis — 22 patients; mild periodontitis — 31 patients; moderate periodontitis — 19) as well as 25 persons with healthy periodontium (the control group). A conventionally accepted clinical and instrumental examination revealed that all the patients with inflammatory periodontal diseases featured the most typical signs of periodontal issues: in the crevicular fluid — an increase in the levels of pro-inflammatory cytokines and chemokines (IL-1β, IL-6, IL-17, TNF-α), a decreased level of anti-inflammatory cytokine IL-1RA, and an increased concentration of vascular endothelial growth factor (VEGF). A ROC analysis showed that the increased content of VEGF, IL-8, IL-1β, IL-6 in the crevicular fluid is of a high diagnostic value in terms of detecting initial inflammation in the gum tissues, whereas the biomarkers pointing at periodontal destructive changes included high levels of VEGF, TNF-α and chemokines.

KEYWORDS — gingivitis, periodontitis, cytokines, chemokines, vascular endothelial growth factor, crevicular fluid.

RELEVANCE

The diagnostics of periodontal diseases relies currently on the results of clinical and radiological studies. Moreover, molecular genetic research and advanced computer technologies are gaining more and more of a foothold in diagnosing periodontal pathology. Unlike traditional laboratory technologies the newly developed diagnostic methods in question offer no sufficient evidence confirming their respective effectiveness. However, a number of studies have already been held, which serve evidence to the need of introducing a number of periodontal pathology biomarkers into the dental clinical practice [1, 10–12]. Biomarkers characterizing the periodontal status are considered to be the key to personalized medicine. As far as diagnostics and treatment of oral diseases are concerned, dental practice is currently facing a need to switch from traditional technologies to approaches based on precision medicine [2].

Given immunoglobulins, lysozyme, T-and B-lymphocytes (ratio 1/2,7), electrolytes, etc. contained in gingival crevicular fluid (GCF), this is seen nowadays as an essential factor of oral cavity local protection. Certain authors (N. Brill, V. Krasse, 1958; G. Cimasoni, 1983) claim that the formation of GCF is associated with an increase in the gum tissues permeability during inflammation. The intensity of the crevicular fluid release depends on the connecting furrow epithelium filtration factor, and especially by the pressure gap between the interstitial and the crevicular fluid. From this stance, the use of qualitative and quantitative GCF indicators for diagnosing periodontal issues of inflammatory origin appears well-grounded and promising.

The most common inflammatory periodontal diseases (IPD) are considered currently not only as an effect of the gum tissue response to the biofilm developing due to plaque bacteria, yet also as something taking place through altered epithelial-immune interaction, cytokines being the major agents of this process [3, 4, 5]. An increase in their content in the GCF is due to an influx of neutrophil cells, macrophage monocytes, T-and B-lymphocytes from the peripheral blood into the gingival sulcus in case of inflammatory periodontal diseases. Cytokine production of each of the gingival sulcus cells groups in case of IPD is aimed at both at immune protection and at the destruction of tissues in the inflammation focus. These cells, which make up the focus of local inflammation, lead to the accumulation of numerous cytokines in the contents of the gingival furrow, primarily such as IL-1β, IL-6, TNF-α, IL-8, MCP-1, etc. At the initial stage, there is an accelerated destruction of epithelial cells and collagen structures developing in the inflammation focus. Further on, the inflammation leads to the bone tissue resorption in the interalveolar septae. The GCF cytokine profile is considered one of the

features indicative of the IPD activity and severity [6]. Changing levels of cytokines in the GCF was found to allow adjusting the complex treatment of IPD and identifying the directions of personalized therapy, evaluating the treatment effectiveness and forecasting the disease course [7, 8, 9]. The results of studying the GCF cytokine profile, however, remain diverse and contradictory.

Aim of study:
evaluation of the diagnostic value of crevicular fluid immunoregulatory biomarkers in case of inflammatory periodontal diseases.

Methodology:
the study relied on data from the respective literature focusing on the IPD etiopathogenesis: Elsevier Science, PubMed Central, ClinicalTrials.gov, MedlinePlus.

MATERIALS AND METHODS

The study involved 97 patients aged 21 to 55 suffering from inflammatory periodontal diseases (gingivitis — 22; mild periodontitis — 31; moderate periodontitis — 19) as well as 25 people with healthy periodontitis (the comparison group). Groups of patients with inflammatory periodontal diseases included the patients who featured typical signs of periodontal damage diagnosed through a conventional clinical and instrumental examination. The comparison group included persons with no signs of periodontal pathology. The exclusion criteria were: concomitant diseases of the digestive system in the acute phase; diabetes mellitus; severe concomitant diseases; the patient's refusal.

The study was carried out at the dental clinic at the S.R.Mirovtsev University Clinical Hospital (part of the Clinical Center of the V. I. Razumovsky Saratov State Medical University) with a prior approval of its Committee for Bioethics.

The focus of research was GCF. After cleaning the teeth from plaque, they were isolated from saliva with cotton rolls and dried. The material from the gingival cavity and / or periodontal pocket was taken with special targets made as paper, absorbent, sterile endodontic pins (Absorbent Paper Points, No. 25). Using tweezers and a packer, two pins were immersed sequentially in the gingival cavity (periodontal pocket), whereas after impregnation they were both put in an Eppendorff-type test tube containing 1000 μl of 0.155 M sodium chloride solution and 0.2% ProClin 300 series biocide [8]. GCF samples with a dilution of 1:200 were frozen at -40° C and stored until the analysis. The concentration of IL-1β, IL-6, IL-17, TNF-α pro-inflammatory cytokines, IL-8, MCP-1 chemokines, the IL-1RA anti-inflammatory cytokine and the VEGF in the GCF samples was identified by enzyme immunoassay using commercial reagent kits (Vector-Best, JSC; Novosibirsk, Russia). The statistical processing of the obtained data was performed using the Statistica v. 6.0 set of software. The results of cytokines quantitative analysis are presented as a median with a quarter-scale (25–75 percentile). The statistical analysis of the results obtained from studying cytokines in the compared groups was carried out relying on the Mann-Whitney U-test, graphical analysis of nonparametric statistical indicators and ROC analysis.

RESULTS AND DISCUSSION

Each of the selected groups of patients with IPD featured specific indicators of the periodontal complex index evaluation (Table 1).

The groups of patients suffering from gingivitis and periodontitis had high medians of the OHI-s, PMA and SBI indices associated with inflammation. An increase in the periodontal pockets depth and the Russel periodontal index, which are typical of a high volume of destructive changes in the periodontal complex, were observed in groups where patients had periodontitis of mild and moderate severity.

A comparison of the cytokine study results in the patients divided into groups, revealed an increase in all proinflammatory cytokines and chemokines in cases with the IPD, and a decrease in the level of anti-inflammatory cytokine, IL-1RA, and an increase in the VEGF concentration (Table 2.3 and Figure 1.2).

There was a direct relationship identified between increased content of pro-inflammatory cytokines/chemokines and the severity of the inflammation clinical manifestations. In case of gingivitis, for instance, as well as in case of mild and moderate periodontitis, the respective GCF featured an increased content of proinflammatory cytokines/chemokines IL-1β, IL-6, IL-8, IL-17, TNF-a/IL-8, MCP-1, and a decrease in the IL-1RA levels. The most meaningful factor in the development of a primary immune response to the introduction of periodontal pathogenic microbiota is IL-1β, whose content in the GCF goes up as soon as at the early stages of inflammation. Increasing levels of pro-inflammatory cytokines/chemokines, as well as VEGF in the GCF of patients belonging to the groups with mild and moderate periodontitis pointed at developing degradation of epithelial cells and collagen fibers of connective tissue further passing onto periodontal support tissues, which means it was associated directly with destructive issues affecting the periodontal complex. The group of patients with moderate periodontitis were found to have the highest PI index and the content of IL-6, IL-8, IL-17, TNF-α, IL-8, MCP-1, and VEGF in the crevicular fluid.

Table 1. *Index evaluation of the periodontal tissues status in patients with IPD*

Examined groups	Index score indicators				
	OHI-S (Green J.C., Vermillion J.R., 1964)	SBI (Mühlemann H.R., Son S., 1971)	PMA (C. Parma, 1960)	PI (Russel, 1956)	Periodontal pocket depth, mm
Gingivitis (n=22)	2 (1,8;2,2)	1,5 (1,4; 1,6)	22,1 (21,4; 26,3)	1 (0,9; 1,55)	-
Mild periodontitis (n = 31)	2,2 (2,1; 2,4)	2,3 (2,1; 2,5)	50,3 (46,7; 52,8)	3,6 (3,4; 3,9)	3,4 (2,7; 3,6)
p-level	0,2	0,015	0,002	0,002	–
Moderate periodontitis (n = 19)	2,6 (2,5; 2,8)	2,5 (2,3; 2,7)	70 (64,3; 77,1)	5,3 (4,9; 5,4)	4,3 (4; 4,8)
p-level	0,2	0,002	0,008	0,001	0,001
Comparison group (n = 25)	1,3 (1,2;1,4)	–	–	–	–

Table 2. *Results of studying the cytokine profile of crevicular fluid in patients with IPD*

Indicator (pg / ml)	Examined groups			
	Healthy (comparison group)	Gingivitis	Mild periodontitis	Moderate periodontitis
	Me [Q1; Q3]	Me [Q1; Q3]	Me [Q1; Q3]	Me [Q1; Q3]
TNF-α	1,58 [1,3;1,9]	1,5 [1,4;1,9]	6,7 [4,5;8,9]*	13,9 [12,8;14,9]*
IL-17	7,45 [6,1;10,8]	18,1 [12,6:21,0]*	15,5 [12,8;22,1]*	28,4 [26,6;33,7]*
IL-6	0,17 [0,1;0,9]	2,9 [1,7;3,4]*	4,2 [2,1;5,6]*	9,4 [8,1;11,3]*
IL-1β	4,9 [2,7;5,3]	10,3 [9,6;11]*	19,6 [11,6;23,6]*	16,2 [13,6;20,1]*

** difference between the comparison group and patients with IPD (Mann-Whitney U test) with a confidence level exceeding 0.95.*

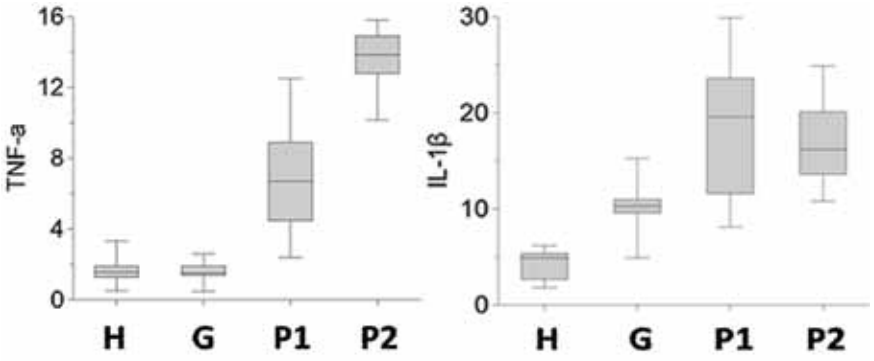

Fig. 1. *The range of TNF-a and IL-1β levels in the crevicular fluid: H — comparison group, G — gingivitis, P1 — mild periodontitis, P2 — moderate periodontitis*

Table 3. *Results of studying the level of chemokines, IL-1RA and VEGF in the crevicular fluid in patients with IPD*

Indicator (pg / ml)	Examined groups			
	Healthy (comparison group)	Gingivitis	Mild periodontitis	Moderate periodontitis
	Me [Q1; Q3]	Me [Q1; Q3]	Me [Q1; Q3]	Me [Q1; Q3]
IL-8	58,7 [23,6;69,5]	75,7 [55,9;189,7]*	122,4 [95,3;173]*	248,2 [213,0;312,7]*
MCP-1	28,2 [21,2;34,4]	26,7 [21,5:31,7]	110,0 [84,1;135,7]*	242,5 [199,1;265,7]*
IL-1RA	3724,7 [2900;4303]	3065 [2772;3478]*	1940 [1559,7;3750]*	2306,2 [1506,0;2759,0]*
VEGF	9,4 [4,9;12,0]	25.1 [18,9;27,8]*	34.5 [30,9;42,7]*	33,5 [27,8;38,0]*

** difference between the comparison groups and patients with IPD (Mann-Whitney U test) with a confidence level exceeding 0.95.*

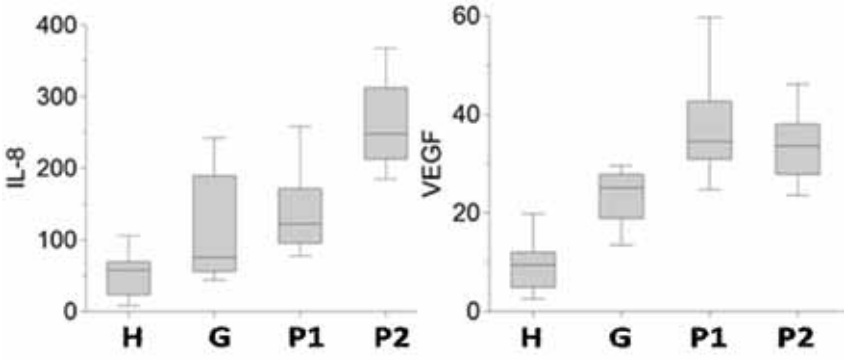

Fig. 2. *The range of IL-8 and VEGF levels in the crevicular liquid: H — comparison group, G – gingivitis, P1 — mild periodontitis, P2 — moderate periodontitis*

An analysis of ROC-curves showed that the increase in the GCF-content of the group of mediators related to immunoregulatory processes is associated with clinical manifestations of inflammatory and destructive changes. Assuming a nonparametric distribution, a confidence level of 95%, including the cut-off value in the positive classification, the increase of VEGF, IL-8, MCP-1, IL-1β, TNF-α, IL-6 in the GCF appears of a reliably high diagnostic value.

Significant binary classifiers were considered those values where the AUC (the area under the ROC curve) was above 0.7 (below 0.3 at a negative dependence) at a significance level of lower than 0.05 when testing the hypothesis stating that the area under the curve was equal to 0.5 (Aundersurve, AUC). Fig. 3–5 offer a view of the respective results.

odontal tissues. During that, biomarkers like VEGF, IL-8, IL-1β, and IL-6 proved of diagnostic value when it came to evaluating the inflammatory process at in the early stages of the disease in case of gingivitis. This proves once again the role of IL-1β as a diagnostic marker of initial inflammation in the gum tissues in response to the plaque bacteria biofilm. High levels of VEGF, chemokines, and TNF-α are to be seen as further biomarkers of inflammation-induced changes that affect the supporting periodontal tissues and lead to the alveolar bone resorption. At thresholds of VEGF — 30.6 pg/ml, MSR-1 — 178.9 pg/ml, IL-8 — 185 pg/ml, and TNF-α — at 11.7 pg/ml, biomarkers reveal sensitivity and specificity from 1.8 to 1.96, which serves proof to their potential use as a group of indicators that allow differential diagnostics of

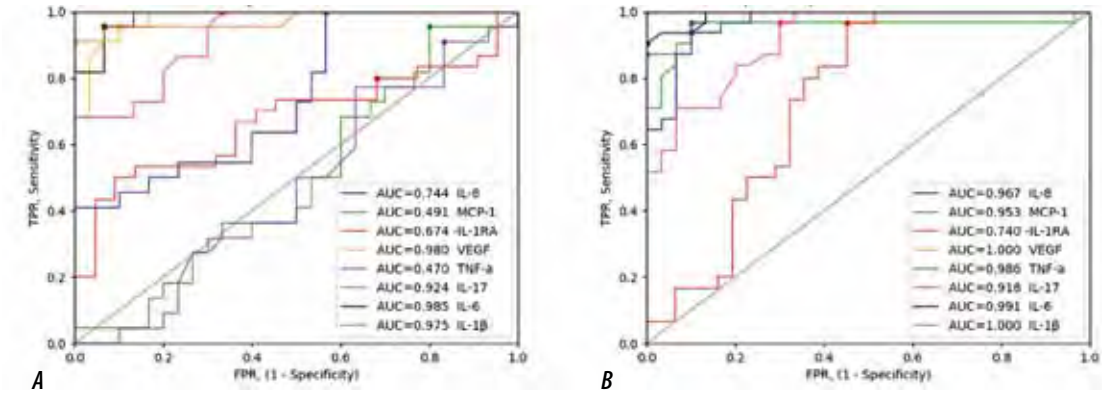

Fig. 3. *ROC curves of the following groups: A — the comparison group and that of patients with gingivitis; B — the comparison group and patients with mild periodontitis*

In patients with mild and moderate periodontitis, the diagnostic value resided in almost the entire group of identified indicators, except IL-1RA and IL-17. That said, an increase in the contents of VEGF, IL-8, MCP-1, IL-1β, TNF-α, IL-6 in the GCF points at a local inflammatory process at the level of periodontitis and gingivitis, as well as monitoring the inflammation passing further to destroy the periodontal ligament and bone tissue.

As of today, there has been a protocol designed for examining patients with IPD. However, diagnosing this pathology at the initial stages still remains

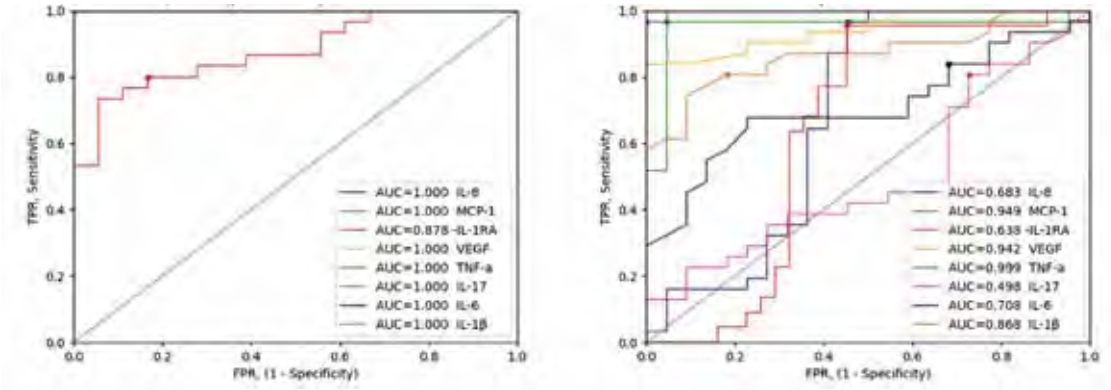

Fig. 4. *ROC curves of the following groups: A — the comparison group and that of patients with moderate periodontitis; B — patients with gingivitis and mild periodontitis*

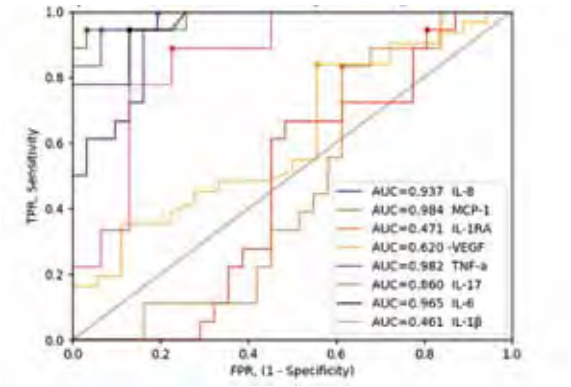

Fig. 5. *ROC curves of groups of patients with mild and moderate periodontitis*

an issue. Patients rarely consult a doctor at the onset of the disease due to poor manifestation of clinical symptoms. Such a latent course of IPD does not allow diagnosing the disease in due time, nor does it facilitate any preventive and treatment measures, as well as supportive therapy. The present study allows proving the diagnostic value of methods employed to obtain and quantify a group of immunoregulatory cytokines in the GCF as biomarkers of early inflammation manifestations affecting periodontal tissues, as well as predictors and indicators to be used for predicting the progression of pain and the development of osteodestructive periodontal changes. This makes it possible to recommend using the quantitative determination of a group of immunoregulatory mediators (VEGF, IL-8, MCP-1, IL-1β, TNF-α, IL-6) in the GCF as personalized biomarkers when developing forecast regarding the disease course as well as the effectiveness of the treatment for IPD. Further developments in the field of saliva proteomic analysis and GCF would pave the way towards new diagnostic tools and personalized medicine. However, their application in dentistry will depend on them being used in daily clinical practice.

CONCLUSION

1. Identification of the immunoregulatory biomarkers levels in the GCF, combined with the results of clinical, laboratory, microbiological, functional and X-ray studies, allows diagnosing the intensity of the damage affecting periodontal tissues, as well as the effectiveness of rehabilitation measures when dealing with patients suffering from inflammatory periodontal diseases.

2. The dynamics of the changes to be observed in the level of immunoregulatory biomarkers contained in the crevicular fluid in case of IPD serves a reliable reflection of the severity of the damage affecting the structural and functional properties of periodontal tissues. As could be seen from the ROC analysis, early stages of inflammation in the gum tissues feature an increase in the contents of VEGF, IL-8, IL-1β, IL-6 in the crevicular fluid, while a high level of VEGF, TNF-α and chemokines in the GCF are indicative of inflammation progress and destructive issues affecting periodontal tissues.

3. The development and shaping of reference intervals related to qualitative and quantitative immunological, physical, chemical, microbiological indicators of the crevicular fluid in case of inflammatory periodontal diseases of various severity, if projected onto the patient's dental status might allow objective evaluation of the degree of disturbances in the oral homeostasis, as well as the imbalance of specific, non-specific factors of local immunity and cytokine profile.

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